Preserving organ function of marginal donor kidneys

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Chapter 1

Introduction and rationale for the thesis

Marginal organ donors can be arbitrarily defined as patients of advanced age and/or those donors who have above-average concomitant morbidity such as impaired renal function, cardiovascular disease, hypertension, or diabetes mellitus. In addition, organs recovered after cardiocirculatory death are also usually considered marginal donor grafts.[1] Kidneys derived from marginal donors may have an impaired posttransplant organ function, with an elevated risk of developing delayed graft function. Also, the risk of primary non-function will be increased. Although many marginal organs will eventually show acceptable function, long-term graft survival can be sub-standard as well.[2-4] In the early days of deceased donor kidney transplantation (1960s through 1980s), donor selection was such that only high-quality organs were considered for transplantation. Not only were waiting lists significantly shorter due to fewer medical indications for a renal transplant, but also were the typical donors young men in their early twenties who had suffered serious cerebral injury after a traffic accident. As a result of increased traffic safety, this category of ICU patients has become relatively rare in the last few decades.[5,6] Aditionally, the practise of early neurosurgical decrompressive craniectomy after traumatic cerebral injury has recently become more common, which is likely to result in a lower number of ICU patients who will eventually meet legal criteria for brain stem death.[7] Lengthening waiting lists in combination with a quickly decreasing pool of "optimal" deceased donors after brain death have urged the transplantation community to accept more kidneys which only a couple of decades ago would not have been considered suitable grafts, such as renal transplants from expanded criteria donors and donors after cardiac death. The various types of organ donors are briefly described in the section below.

Living donors were the first patients from whom kidneys were successfully transplanted, starting with the famous Boston twins in the 1950s.[8] A kidney donated by an identical twin will not require any immunosuppressive therapy in the recipient sibling, and as a result such rare cases in which only one half of a pair of indentical twins developed end-stage renal failure comprised the first serious and often successful attempts at renal transplantation in humans. In the decades that followed, methods were developed to partially suppress the immune response, first by means of total body irradiation, and later by increasingly refined and specific pharmacological agents. This has made tissue-type incompatible kidney allotransplantation possible from living-related and living-unrelated donors.[9] Also, the advent of adequate immunosuppression quickly paved the way for transplantation of kidneys recovered from deceased donors, which can be categorized as follows:

Donors after brain death, also known as heart beating donors, are those ICU patients who have sustained irreversible cerebral injury and meet the legal criteria for brain stem death, which were first described by the Harvard ad hoc committee on brain stem death in 1968 in Boston, MA, USA.[10] Brain death can be the end result of either traumatic injury, or a cerebrovascular event that led to cerebral ischemia and/or compression due to bleeding. Organs recovered from donors after brain death are perfused with the donor's own oxygenated circulation until the moment of aortic clamping in the operating room and systemic cold perfusion with one

of several cold preservation solutions. Although the pro-inflammatory and pro-coagulatory state of brain death itself has a well-documented detrimental influence on donor grafts,[11] organs that are recovered from donors after brain death sustain only minimal amounts of warm ischemic injury. Donors after brain death can be further sub-divided into *standard criteria donors* and *expanded criteria donors*. The latter category is usually defined as donor age ≥60, or donor age between 50 and 60, with at least two of the following additional donor characteristics: (1) history of hypertension, (2) cerebrovascular cause of death, (3) pre-retrieval serum creatinine >132 μmol/l.[12] In the original publication by Metzger *et al*, who developed this definition, the authors do not mention whether their definition also applies to donors after cardiac death. In subsequent publications by the same and other groups, no uniform choice is made as to whether all donors in the latter category are to be considered expanded criteria donors, or only those that meet the definition should be included. Alternatively, many groups implicitly assume that expanded criteria donors can only be donors after brain death. *Donors after cardiac death*, also called donors after cardiocirculatory death or non-heart beating donors, are a heterogenous group of deceased donors who have one characteristic in common: Organs are recovered after cessation of spontaneous circulation due to cardiac death. Therefore, death is declared on classic cardiocirculatory instead of neurologic criteria and, as a result, most donors after cardiac death were either not legally brain dead, or their neurologic status was unknown at the moment of cardiac death. Donors after cardiac death can be further sub-divided into four categories, which were defined at the first meeting on non-heart beating donation in 1995 in Maastricht, The Netherlands.[13] Chapter 2 discusses those four different types and their individual characteristics in detail.

This thesis comprises clinical and pre-clinical studies that aim to quantify the impact that donor characteristics have on posttransplant outcome, and to investigate the effect of interventions before or during organ preservation which might better conserve organ quality prior to transplantation. In addition, two studies aim to predict aspects of transplant outcome by measuring biomarkers in donor plasma and in machine preservation solution, or by assessing machine perfusion characteristics. Although the findings of these studies may pertain to all types of donor kidneys, they are particularly applicable to renal grafts recovered from marginal donors. As outcome of such transplants is often sub-standard, any additional information on organ quality, as well as measures that will better preserve graft function are most relevant for marginal kidneys.

Chapter 2

Donation after cardiac death

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ABSTRACT

With increasing numbers of patients on the waiting list for organ transplantation, many centers are revisiting donation after cardiac death (DCD) as a tool to enlarge the deceased donor pool. Early scepticism has changed to careful enthusiasm, as first long term results after DCD kidney transplantation are promising. To date, extrarenal DCD organs are also considered a serious option to close the gap between organ supply and demand. However, warm ischemic injury leads to potentially more organ dysfunction compared with grafts derived from brain dead donors. Minimizing graft damage is one of today's challenges in DCD donor management and organ preservation. This review discusses mechanisms of warm ischemic injury, potential new approaches to improve posttransplant results, and several persistently controversial issues in DCD. In addition, we provide an overview of current DCD protocols and up-to-date evidence on selection criteria, organ preservation, and clinical outcome after transplantation of various types of DCD organs.

INTRODUCTION

The concept of donation after cardiac death (DCD) is not new. In the early days of organ transplantation, all deceased donor grafts were retrieved from donors who had suffered cardiac death.[14-16] When legal definitions for brain death became available in the 1960s of the last century,[10] most centers established transplant programs based on organ retrieval from donation after brain dead (DBD), thus avoiding the warm ischemic damage that DCD donor organs by definition have sustained.[17]

Organ donation, however, has become a victim of its own success. In the last decades, indications for transplantation have become broader, whereas donor organ availability did not increase substantially. Partially due to improved traffic safety regulations, the number of DBD organ donors has dropped: In the Eurotransplant region the relative amount of donors with cerebral trauma decreased from 43% in 1990 to 35% in 2005.[18,19] Attempts to improve the willingness of the public to donate their organs after death have been only marginally successful. All these factors contribute to an ever increasing number of patients on the waiting list. Within Eurotransplant alone, on December 31, 2005, more than 15,000 patients were waiting for an organ. Less than 6,000 transplants were performed in that same year and almost 1,400 patients died while on the waiting list.[19]

In an effort to enlarge the donor pool, living donation has made a valuable contribution to kidney transplantation programs, and living split-liver donation is a promising method for the future in liver transplantation.[20,21] However, such programs will never yield sufficient new donor organs to bridge the gap between supply and demand.

To date, many centers are revisiting DCD in order to enlarge the deceased donor pool. This is a logical step, for the potential pool of these donors is many times larger than the amount of available DBD donors.[17,22,23] In the late 1980s and early 1990s, a few hospitals had already re-introduced DCD protocols. The group from Maastricht, led by Kootstra, was one of the pioneering centers.[24] In 1995, at the first international workshop on DCD donors in Maastricht, consensus was reached about donor management protocols and four different categories of DCD donors were defined (Table 1).[13] Ever since, the practice of DCD donation has increasingly become a part of transplant programs all over the world.

Category	Description	Organ recovery
I	Dead on arrival	Uncontrolled
II	Unsuccessful resuscitation	Uncontrolled
III	Awaiting cardiac arrest (withdrawal of treatment)	Controlled
IV	Cardiac arrest while brain dead	Uncontrolled

Table 1: Maastricht classification of donors after cardiac death

With increasing numbers of grafts that have suffered from prolonged warm ischemia, maintenance of organ viability has once again become an important factor to preserve current high standards for functional outcome and long term survival after transplantation. The amount of injury differs for the various DCD donor categories.[13] Category III donors are most widely used, since the duration of warm ischemia (WI) is known and usually short. In addition, organ recovery can be planned in advance. Nevertheless, the time interval between withdrawal of treatment and cardiac arrest in the potential donor may account for additional WI injury due to low oxygenation and organ hypoperfusion. This period is usually not included in calculations of total WI time, but it is likely to be relevant to appreciate the real ischemic insult that a particular organ has sustained. The length of this so-called agonal phase varies widely between individual donors, and many different upper limits for acceptable donation are in use, depending on which organs are to be procured. While for example in The Netherlands a maximum period of two hours is considered acceptable for kidney donation,[25] US guidelines recommend no more than 60 minutes.[26] Since ischemic injury accumulates as a continuum, influenced by a multitude of factors, setting an evidence based cut-off value for the maximum length of the agonal phase remains difficult. Suntharalingam *et al*. have recently conducted a comprehensive multicenter study to identify clinical parameters that independently predict the timing of death following treatment withdrawal. Their data show that younger age, higher FiO_2, and the mode of ventilation (no pressure support vs. pressure support) are independently associated with a shorter agonal phase before cardiocirculatory death.[27] These are improrant findings, as they may allow better identification of patients suitable for DCD and facilitate timing of organ retrieval. Various guidelines are in use for the maximum acceptable duration of true warm ischemia (commonly defined as the interval between a mean arterial pressure below 60 mmHg and initiation of organ perfusion). Most up-to-date evidence shows that for the liver, a WI time above 20-30 minutes, and for the kidney a WI time longer than 45-60 minutes is associated with increased complications posttransplant.[28]

In some countries donation after withdrawal of treatment is illegal. As a result, transplant programs have to rely solely on uncontrolled DCD, in which the average WI time is considerably longer. However, uncontrolled DCD may have one advantage over category III donors: Serious brain injury is associated with a significant pro-inflammatory and pro-coagulatory response in the donor, which has a negative effect on organ quality and increases the risk of immunological complications posttransplant.[11] Most controlled DCD donors have sustained irreversible cerebral injury. As a result, their organs may suffer more from negative immunological and coagulatory effects than grafts derived from uncontrolled DCD donors, whose primary medical condition is usually not neurologic. In renal transplantation, the detrimental effect of delayed graft function (DGF) on graft survival appears to be more pronounced in kidneys derived from brain injured donors, versus organs coming from uncontrolled DCD donors.[29] These data suggest that WI plus profound cerebral injury could account for a different, more detrimental form of DGF than observed in uncontrolled DCD kidneys that have sustained only WI.

Mechanism of warm ischemic injury

Tissue ischemia leads to a cascade of cellular injury and repair responses. Lowering organ temperature to 0–4ºC will slow down such responses, although accumulation of injury will continue at a rate of ~10% from normal.[30] For this reason, hypothermic organ preservation cannot be extended beyond certain time constraints, since cold ischemia will keep the graft in an acceptable condition for only a limited period.

The onset of ischemia immediately impairs oxidative metabolism. This leads to depletion of ATP, an increase in anaerobic glycolysis, and inhibition of Na^+/K^+ ATPase. Membrane transport mechanisms will slow down, causing intracellular accumulation of water and ions which results in cell edema and dysruption of the cytoskeleton. Impaired oxidative metabolism triggers formation of radical oxygen species (ROS) that have a direct detrimental effect on the cell. ROS will also facilitate production of other free radicals such as nitric oxide (NO), further disrupting the cytoskeleton. Anaerobic glycolysis lowers the intracellular pH due to synthesis of lactic acid, which negatively influences cellular homeostasis. In addition, hypoxia will inhibit cytoprotective mechanisms, such as upregulation of heme oxygenase-1 (HO-1) and heat shock protein-70. Impaired cytoprotection will render the graft more susceptible to further ischemic injury.[31] At reperfusion, more injury ensues when damaged endothelial cells express intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, which attract host leukocytes. These leukocytes release more ROS and inflammatory mediators, aggravating cellular injury. Ischemia-reperfusion injury also stimulates antigen-independent, innate immunity via complement activation and Toll-like receptor(TLR)-mediated pathways. Innate immune activation in turn triggers the adaptive immune response, in part through TLR-induced surface expression of CD80 and CD86 on dendritic cells. This will cause early T-cell regulated inflammatory damage to the graft. Adaptive immune activation will also increase the risk of acute rejection. Both, innate and adaptive immune responses eventually contribute to the development of chronic allograft pathology.[32] Recent evidence suggests that ischemia-reperfusion injury is a highly coordinated and specific process mediated by components of both innate and adaptive arms of immunity.[33]

After reperfusion, energy levels in the graft are rapidly restored. This fuels cytoprotective processes, such as formation of HO-1 and vascular endothelial growth factor expression, which protect cells from the host immune attack.[34] A sequence of events follows, initiating repair of endothelial, epithelial, and parenchymal cells. Although mechanisms and rates differ between various cell types, cell differentiation, migration, and proliferation directed by growth factors and molecular signalling pathways play an important role in the repair response.[35]

Novel approaches

DCD grafts are exposed to significantly more ischemia-reperfusion injury than organs derived from donation after brain death (DBD). In general, the most obvious and economic approach

to minimize injury would be to keep WI time as short as possible, and to limit cold ischemic time. Reducing ischemic times by just a few percent is likely to have a much larger impact on outcome than the application of novel technical or pharmacological interventions.[36] Normothermic recirculation and normothermic machine perfusion are sophisticated novel organ preservation techniques, which aim at providing oxygen and nutrients to the graft during organ preservation to better maintain viability and perhaps fuel cellular repair mechanisms.[37,38] In the Hospital Clínic in Barcelona, Spain, a protocol for normothermic recirculation of uncontrolled DCD donors is in use: Before cold storage is initiated, the donor is rewarmed and recirculated for a brief period with normothermic extracorporeal membrane oxygenation via the femoral vessels. The group reports resuscitation of otherwise non-transplantable liver grafts.[39] Donor pretreatment with anti-inflammatory, anti-coagulatory, and other agents, as well as addition of thrombolytic agents to the systemic flush solution could mitigate the initial ischemic insult.[40] However, treatment of a donor-to-be before the legal determination of death is associated with serious ethical concerns. For the near future, finding agents that are beneficial for both, the critically ill ICU patient and his or her potential donor organs may be the most pragmatic approach.

Controversial issues

Apart from ethical concerns about donor pretreatment, one of the largest other controversies in DCD to date surrounds the issue of donor type substitution. A most striking example is the recent situation in The Netherlands (Fig. 1): In a short time period, controlled DCD has become very popular, with exceptional rates approaching 50% of all deceased donor procedures. Surprisingly, this did not result in enlargement of the donor pool. The absolute number of kidney donations and transplants remained approximately the same, whereas the number of procured thoracic organs decreased (source: Eurotransplant annual report 2008). It is very difficult and politically sensitive to pinpoint a single cause for this alarming phenomenon, but it seems plausible that some form of substitution could be involved.[2] A possible mechanism might be that donor families are given a choice between a controlled DCD or a DBD procedure. For the family, the timely withdrawal of treatment followed by cardiocirculatory arrest may be perceived as a more emotionally acceptable way to cope with the loss of a beloved one, even if the patient meets legal brain death criteria or progression to brain stem death is imminent. In addition, current high pressure on ICU beds may add to an eagerness to initiate donation procedures as soon as possible, rather than to wait up to a few days for brain stem death. Also, a lower treshold to perform early decompressive neurosurgical interventions in patients with cerebral injury could have resulted in an absolute decrease in the number of ICU patients who eventually progressed to brain stem death. All these factors together could lead to relatively more DCD procedures, and hence fewer available hearts, livers and pancreata.

Another persistent concern in DCD is the question when exactly a patient may be declared dead from a cardiocirculatory point of view. In order to maintain societial support for DCD it is essential to have transparent policies for the indication to initiate withdrawal of

treatment, and a well defined no-touch period afterwards. Common consensus requires that the physicians who are involved in the cessation of life support and the declaration of death are always strictly separated from the organ procurement team. In addition, the most up-to-date evidence suggests that the declaration of cardiocirculatory death should be no earlier than two minutes after asystole, since autoresuscitation has never been reported after this period.[41] Most protocols to date dictate a no-touch period of 3–5 minutes, although some centers use a 10 minutes interval.[28,42]

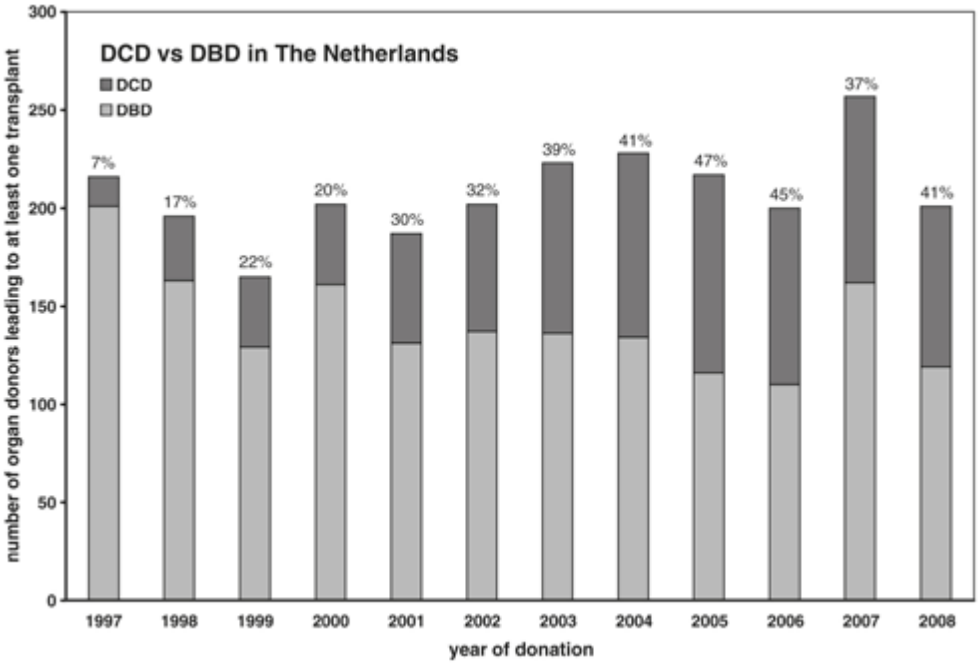

Figure 1: The annual number of DCD and DBD procedures in The Netherlands leading to at least one transplant. Above each bar, the percentage of DCD versus total deceased donor procedures is indicated. From 1997 through 2008, the relative contribution of DCD to the deceased donor pool increased from 7% to more than 40%, whereas the total annual number of deceased donors did not rise. These figures could indicate substitution of DBD for DCD procedures. Source: Dutch Transplantation Foundation annual reports 1997–2008.

The use of extracorporeal membrane oxygenator support after cardiac arrest, as practiced by some centers, may raise paradoxal ethical concerns. If the heart is reperfused with oxygenated blood it will likely resume a normal rhythm, thus potentially affecting the "state of death" that had been declared a few minutes earlier based on cardiocirculatory criteria. Hence, physicians often choose to inflate a thoracic aortic balloon, or administer lidocaine to prevent the heart from resuming activity. Nevertheless, it may be argued that irreversible brain injury has already taken place when cardiac reanimation occurs, provided that a reasonable 2–5 minutes no-touch period was observed after cardiac arrest.[43]

CLINICAL EVIDENCE

The kidney

For kidney recipients, dialysis is always available as a backup in the case of insufficient immediate graft function. Therefore, kidneys were the first organs to be transplanted from DCD donors. Renal grafts comprise by far the largest group of DCD organs actually used for transplantation (Fig. 2a). To date, DCD kidneys of all Maastricht categories are used worldwide, but categories III and II are predominant. Many centers use rapid in situ cooling techniques for category II kidney-only donor management. After unsuccessful resuscitation, both kidneys are perfused with cold preservation solution following insertion of a double-balloon-triple-lumen (DBTL) catheter via the femoral artery. Various protocols are in use for category III donor management. Although DBTL catheter in situ cooling can also be utilized when extrarenal organs are to be procured, rapid laparatomy with aortic cannulation and systemic cold perfusion is nowadays the most widely used technique for the DCD multi-organ donation scenario. Some centers do use DBTL catheter cooling for category III kidney-only donors, however, evidence suggests that rapid laparatomy with aortic cannulation leads to comparable results and fewer technical complications.[44] Category I donors are used to some extent by a few centers, e.g. by the Madrid group in Spain. This center employs strict emergency service protocols to minimize WI time and has a high organ discard rate (57%) due to stringent donor selection criteria. In a country where category III DCD is illegal, this pragmatic approach provides an alternative source of donors to bridge the gap beween organ supply and demand. The group reports 68% delayed graft function (DGF), 6% primary non-function (PNF) and a similar 5 year graft survival (GS) as achieved with DBD kidneys (~75%).[45] However, when interpreting these numbers it should be kept in mind that kidneys have been subjected to an exceptionally strict selection process with a considerable discard rate: The group uses only those donors with a known time between cardiac arrest and initiation of adequate cardiopulmonary resuscitation under 15 minutes, no violence as cause of death, no thoracic or abdominal bleeding injuries, no more than 120 minutes between start of resuscitation and initiation of organ preservation, and the availability of a next of kin within four hours. DCD cat. IV donors including sudden cardiac death after declaration of brain death are a very rare group, for which hardly any isolated data are available.

The question of how to best preserve DCD kidneys has remained unresolved until recently. Many centers embarked on static cold storage (CS), whereas others strongly advocate hypothermic machine perfusion (MP), especially for category II grafts. Retrospective studies suggest a short and long term outcome benefit of MP versus CS.[46] A prospective study conducted in the United Kingdom on MP versus CS for DCD kidneys was terminated early as the investigators expected that it would not show any difference in outcome after transplantation.[47,47] However, the recent large European prospective randomized controlled trial comparing MP with CS preservation showed that MP indeed reduced the risk of DGF with an adjusted odds ratio of 0.57 for all common types of deceased donor kidneys, regardless of whether the graft came from a DCD,

DBD, or expanded criteria donor. In addition, MP reduced the risk of graft failure in the first year posttransplant with an adjusted hazard ratio of 0.52 versus CS.[48] Hence, with this level of evidence and since the incidence of DGF is particularly high in DCD kidneys, MP appears to be the best choice to preserve a DCD kidney graft. Two very recent analyses derived from the same prospective study showed that MP characteristics such as perfusate flow, intravascular resistance and the biomarkers glutathione-S-transferase and heart-type fatty acid binding protein do have some predictive potential for delayed graft function. However, none had any relevant prognostic value for serious complications such as primary non-function and graft failure. Therefore, MP dynamics and perfusate biomarker measurements may help to fine-tune postoperative recipient management (e.g. delay introduction of calcineurin inhibitors), although they should not be used to accept or discard a kidney.[49,50]

Kokkinos *et al*. conducted a comprehensive meta-analysis of currently available clinical data on DCD kidney transplant outcomes. Their study showed that, for all categories pooled, the incidence of DGF has an odds ratio (OR) of 3.64, when compared to DBD kidneys. PNF also occurs more frequently (OR 2.43). DCD kidney recipients tend to stay more days in-hospital after transplantation (OR 4.56). Graft survival of DCD kidneys is generally somewhat inferior to DBD grafts, with ORs of 0.70 at three months and 0.89 at 10 years posttransplant, although this last OR tested non-significant. Acute rejection rates and patient survival posttransplant do not differ from DBD kidney recipients.[51] Snoeijs *et al*. showed that the use of elderly DCD donors was associated with unacceptable clinical outcomes. They concluded that transplantation of 65+ DCD renal grafts cannot be justified without further refinement in their selection, for example, by histological assessment of pretransplant biopsies.[52] In summary, DCD kidneys show an inferior short term function, but seem to have only a mild graft survival disadvantage in the long run, as long as donor age is under 65. Although these data will convince many transplant professionals that introduction of a DCD program can be a safe addition to the deceased donor pool, some consideration should be observed when interpreting long term results. Today, follow-up data of more than 5 years posttransplant are only available for a relatively small number of DCD kidney recipients. These were the patients who received a kidney transplant when DCD was cautiously re-introduced by some centers. Therefore, their grafts may have gone through a much stricter selection process than the average DCD kidney undergoes nowadays. This could bias the long term DCD outcome we are currently looking at, towards a better GS than DCD kidneys transplanted today will show after the same time interval. Ongoing monitoring of long term outcome therefore remains important to keep results in line with current clinical standards.

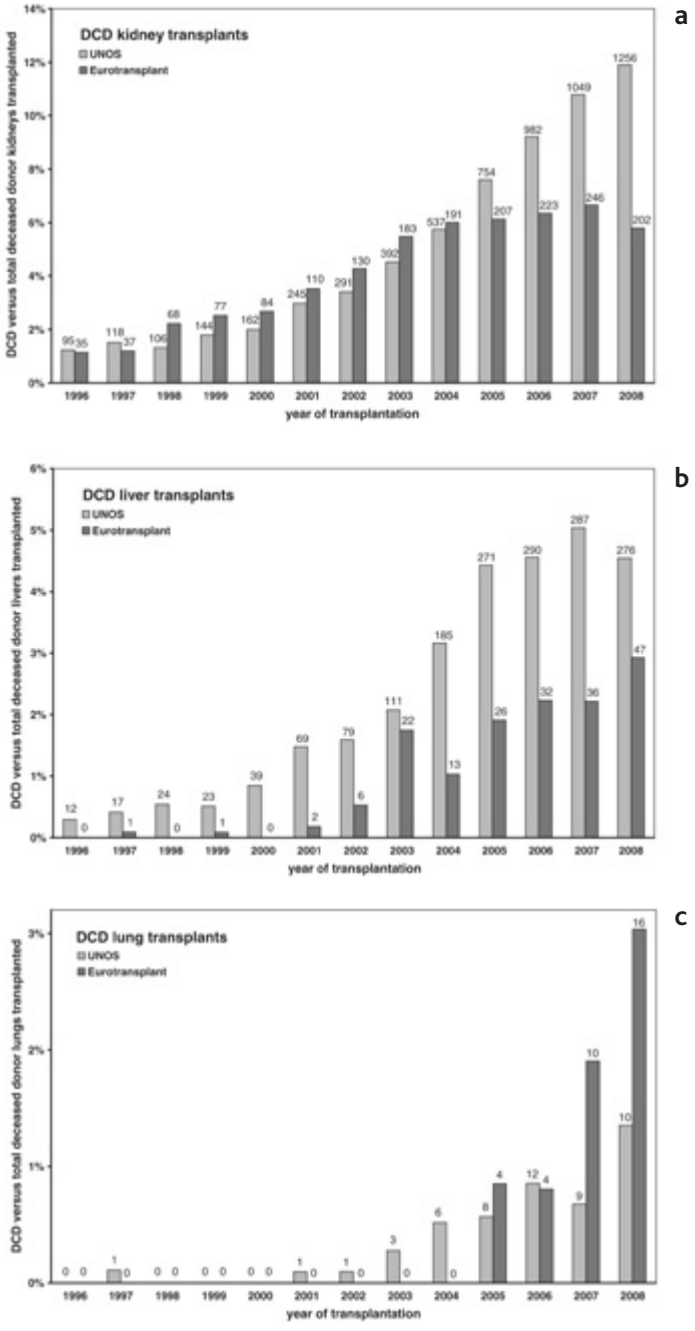

Figure 2 a-c: DCD transplants performed per organ type in the USA and in Eurotransplant (an international organ exchange organization in Europe). Bars represent the percentage of DCD grafts in the total deceased donor transplant volume of this organ type. Above each bar, the actual number of DCD transplants is indicated. Source: UNOS and Eurtotransplant custom data requests.

The liver

In contrast to the kidney, DCD liver transplantation is introduced into programs around the world with much more hesitation. Between 1996 and 2008, 1,683 DCD livers were transplanted in the USA, and 186 in Eurotransplant (Fig. 2b). Due to the lack of life-sustaining replacement therapy, most extrarenal organs undergo a more stringent selection process in order to prevent PNF, which implies retransplantation or death within seven days posttransplant. Many studies have shown that the liver, especially its sinusoidal cells and the biliary system, is less tolerant to ischemic injury than a renal graft.[53] The burden of increased ischemic type biliary complications in DCD livers may account for additional posttransplant morbidity that is not necessarily outlined by basic survival analyses.

To date, all livers are preserved by static CS. Although evidence coming from kidney preservation studies may be extrapolated to the liver as well, MP preservation of liver grafts has not reached the clinic yet. Clinical MP for livers is often considered less feasible due to the more complex system needed to perfuse both the hepatic artery and portal vein, rendering a potential device less transportable.[54] However, if these technical concerns are overcome, MP could be a promising method to enlarge the potential DCD liver pool. In addition, MP may offer the option of in vitro viability testing as a tool to aid decisions on organ quality. The question remains whether MP will help reduce ischemic type biliary lesions.

In a retrospective analysis by Freeman *et al*., overall posttransplant outcome of DCD liver transplants in the USA between 2000 and 2006 (n = 1,007 in their study) was inferior compared to DBD livers: four-year adjusted graft survival was almost 20% lower.[55] Both, Mateo *et al*. and Lee *et al*. have published detailed analyses of DCD liver transplant outcome. Much effort was directed at identifying selection criteria for the acceptance of a DCD liver. From the evidence currently available, it is clear that non-steatotic liver grafts from relatively young DCD donors (≤45 years) with short WI time (≤15 min.), kept on CS preservation for ≤10 hours are safe candidates for transplantation. Interestingly, recipient characteristics had no relevant predictive value for graft survival, as long as the aforementioned criteria were met. GS for this group (84.9% at 1 year; 69.4% at 5 years) was comparable to that of DBD livers.[56,57] To summarize, data currently available suggest that with careful selection of suitable donors, DCD liver transplantation is within reach of everyday transplantation practice and could reduce the number of patients on the waiting list.

The lung

Clinical DCD lung transplantation is a slowly emerging field (Fig. 2c). Approximately one decade ago a few centers started small DCD lung transplant programs. Data derived from animal studies had pointed out that lungs do not rely on arterial perfusion to deliver oxygen for cellular respiration. Since parenchymal cell oxygenation occurs through air spaces, merely ventilating non-perfused lungs will provide sufficient oxygen to prevent serious ischemic

tissue injury.[58] Therefore, pulmonary grafts derived from DCD donors will suffer less from WI compared to other organs, especially when procurement can be planned in advance. In category III DCD, the donor can be rapidly re-intubated and ventilated after the legal five minute no-touch period following cardiac arrest. In an uncontrolled donation setting, lung viability may also be preserved as long as adequate artificial ventilation is started immediately after cardiac arrest.

With only scarce evidence available, DCD lung preservation seems to rely on rapid organ cooling, as soon as ventilation is discontinued. For uncontrolled donors, Steen *et al*. have advocated intrapleural cooling within the intact body, followed by warm ex vivo functional evaluation.[59] However, in a controlled DCD donor, systemic cold flush after rapid aortic cannulation may be sufficient to preserve organ viability.

Although various groups have reported cases or small numbers of successful DCD lung transplants at conferences, only a handful of such series has been published so far. One of the largest studies appeared in 2007, presenting posttransplant outcome of 17 uncontrolled (categories I and II) DCD pulmonary grafts. The authors report that, even with an organ discard of around 87%, the rate of primary graft dysfunction in the recipient (53%) was much higher than in DBD lungs (10–20%). Three year patient survival was 58%.[60] Early results of another series in Australia were recently reported by Snell *et al*. Out of 11 donation attempts, eight Maastricht cat. III lungs were retrieved and successfully transplanted. At the moment of their report, all eight recipients survived for a mean of 311 days with an acceptable early clinical course.[61] In an OPTN database analysis, Mason *et al*. compared outcomes of 36 DCD lung transplants in the USA to average outcomes of DBD lungs. They concluded that DCD resulted in survival up to two years which was at least equivalent to that after DBD.[62] In the University Medical Center Groningen, The Netherlands, a significant DCD lung transplant program exists since 2005. So far, 24 pulmonary grafts retrieved from DCD cat. III donors were successfully transplanted, with an early postoperative course comparable to DBD lungs. (M.E. Erasmus, personal communication, May 1, 2009). In conclusion, DCD has had a minimal impact on lung transplantation so far. However, interest in this new practice is increasing and larger studies presenting outcomes after transplantation are awaited with anticipation.

Other organs

The University of Wisconsin group from Madison, WI has published outcomes of a large consecutive series of DCD simultaneous pancreas and kidney transplants (n = 37). The authors report that 5-year patient, pancreas, and kidney survival was similar to that of DBD transplants.[63] DCD pancreas-only transplants are hardly ever reported, with some rare exceptions coming from Japan. Currently, most DCD pancreatic grafts are used to obtain islets for transplantation.[64]

Transplantation of cardiac grafts derived from DCD donors has remained in a predominantly pre-clinical phase so far. Myocardial vulnerability to ischemic injury would make donor management in the DCD setting challenging.[58] Although the potential donor pool expansion could be interesting,[65] no centers have transplanted DCD hearts on a relevant scale. Clinical cases using a normothermic resuscitation and preservation device have been reported at meetings, but no reliable outcome data have ever been published.[38]

For DCD intestinal transplantation, only scarce data are available. The number of suitable DBD grafts outnumbers the relatively small group of serious candidates for an intestinal allograft. Moreover, small bowel tissue is highly susceptible to WI injury. Therefore, no rationale seems to exist for transplanting intestines recovered from DCD donors.[66]

CONCLUSION

Donation after cardiac death is rapidly earning its place in everyday clinical transplantation practice. Prolonged WI leads to organ injury at various levels, which should be minimized to preserve organ viability. This poses considerable challenges to DCD donor management. In contrast to widespread sceptisism only a few years ago, many centers today have adapted their protocols to incorporate the option of DCD. For the kidney, large series of long term follow-up are now becoming available, with encouraging results. Transplantation of extrarenal organs is gaining acceptance, with livers and lungs as the most serious candidates. Also, there is increasing evidence that DCD pancreata are likely to perform equally well compared to those recovered from DBD donors. However, long term clinical outcome data are very scarce, and more evidence has to become available before these organs can be considered to safely reduce the number of patients on the waiting list.

Chapter 3

The influence of deceased donor age and old-for-old allocation on kidney transplant outcome

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ABSTRACT

Background

Transplantation of older deceased donor kidneys is gaining wide acceptance in most countries. Many previous studies have concluded that advanced donor age negatively impacts posttransplant outcome, but detailed data on the extent to which a few years increase in donor age will influence early graft function and graft survival (GS) are scarce.

Methods

We used the Organ Procurement and Transplantation Network database (cohort 1994–2006, n = 99,860 recipients) to evaluate the effect of deceased donor age on posttransplant results, and to obtain regression models which are relevant to guide clinical organ allocation policies. In addition, we simulated the effect that old-for-old allocation would have on transplant outcome.

Results

In the context of other risk factors, donor age increased the risk of delayed graft function (DGF) and graft failure with odds/hazard ratios of 1.02 and 1.01, respectively. Absolute DGF risk increased by 0.35–0.37% and GS decreased with each year increase in donor age. Kidney discard rates in the USA increased with donor age, up to 66.9% for 65+ donors. In our simulation, we found that old-for-old kidney allocation would have no large impact on overall renal transplant outcome.

Conclusions

This study shows that donor age strongly influences posttransplant outcome, not only in the upper extremes, but for the whole range of donor ages ≥11. Implementation of old-for-old kidney allocation is likely to be safe. Such a policy could reduce waiting time for aged candidates, but it will not necessarily improve overall kidney transplant outcome.

INTRODUCTION

With increasing numbers of patients on the waiting list, transplantation of kidneys from sub-optimal donors has gained wide acceptance in most countries. Older donors, expanded criteria donation (ECD), and donation after cardiac death (DCD) have nowadays become important sources of kidney grafts.[18,67-69] Various studies indicate that organs derived from such elevated-risk donors can be used successfully, provided that careful selection criteria are employed. Nevertheless, in most large registry analyses advanced donor age remains one of the most important risk factors for inferior posttransplant outcome.[70-75] Although a number of previous studies have superficially assessed the impact of donor age, detailed data on the extent to which a few years increase in donor age will influence early graft function and graft survival are scarce.

Within Eurotransplant, an international organ exchange organization in Europe, a well-established old-for-old allocation program exists since 1999.[2] In this kidney exchange program, 65+ deceased donor grafts are allocated to non-immunized recipients of 65 years and older, employing only ABO blood group matching and a policy to keep preservation times short. Results of this program and other senior-recipient organ exchange programs are encouraging, with a higher utilization rate for older donor kidneys, shorter waiting times for older patients, and reduction of the number of older patients on the waiting list. Overall long term outcomes after transplantation appear not to be negatively affected by this policy.[76]

Our current analysis focuses on the influence of deceased donor age on renal transplantation in the USA, and addresses the question whether old-for-old allocation is safe. Aims of the study were to obtain regression models that show in detail the effect of donor age on short- and long-term outcome, and to simulate kidney graft survival rates if an old-for-old kidney allocation program were implemented in the USA.

METHODS

Study population

A January 10, 2007 extract of the Organ Procurement and Transplantation Network (OPTN) database was used. The study population consisted of deceased donor single-kidney recipients who were transplanted from January 1, 1994 through December 31, 2006. Only transplants from donors aged ≥11 years were included in the analysis. We chose 1994 as lower boundary of this cohort, since several important variables were not collected before this year, and also because postoperative care before this year would be too different from today's regimen. The upper limit was 2006 as database completeness for transplants performed thereafter was still too low at the time of analysis.

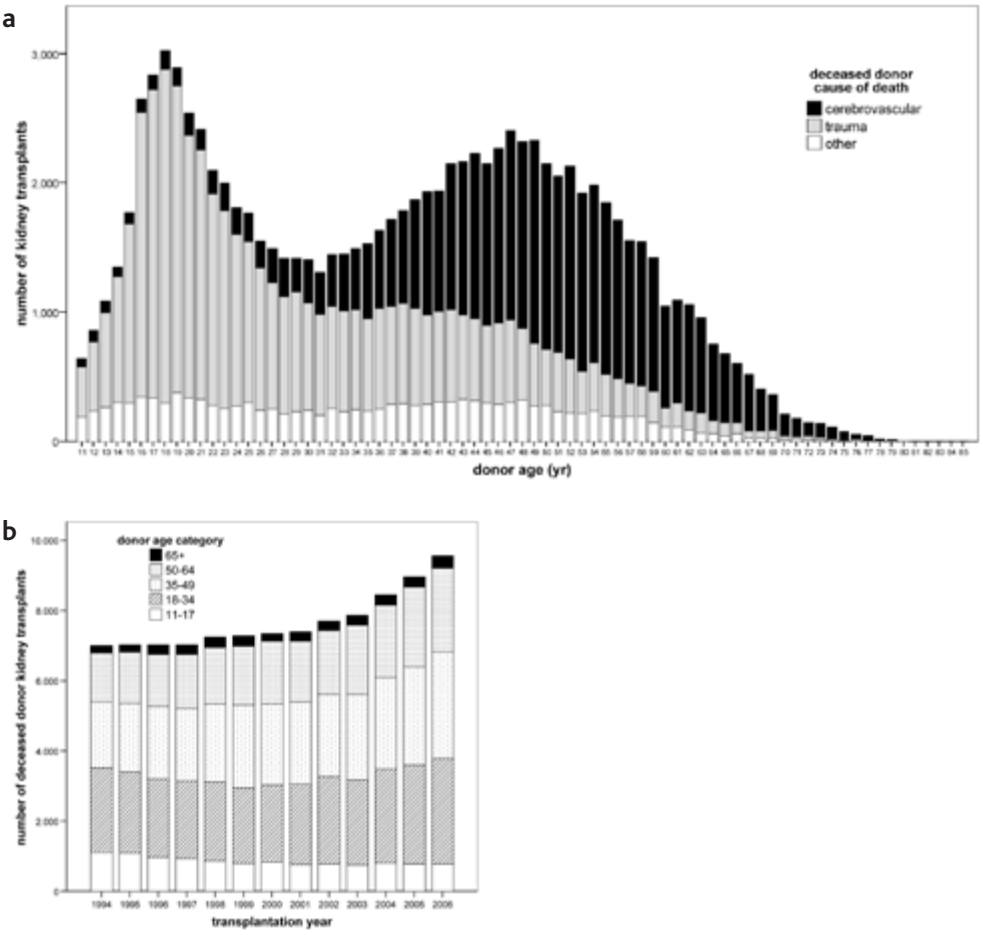


Figure 1: a) Distribution of deceased donor kidney transplants (cohort 1994–2006) per donor age, stratified into the two major causes of death; **b)** Total number of deceased donor kidney transplants per year, distributed over five different donor age categories.

Endpoints

Endpoints for short-term outcome after kidney transplantation were delayed graft function (DGF) and primary non-function. DGF was defined as any dialysis requirement in the first week after transplantation. As reliable data on primary non-function could not be easily derived from the OPTN database, graft loss within three months posttransplant was used as a surrogate. Graft survival (GS) up to 10 years posttransplant served as endpoint for long-term outcome.

Statistical method

Donor, transplant, and recipient demographics were calculated for the study cohort, and plotted in graphs showing causes of death and the number of transplants per year. For each year between 1994 and 2006, kidney discard rate was visualized as the percentage of kidneys actually transplanted from all deceased donors recovered in various donor age categories. The correlation between donor age and recipient age was calculated by Pearson's method. A binary logistic regression model was employed to identify independent donor-, preservation-, and recipient-related risk factors for DGF and for graft loss within three months posttransplant. Cox regression models examined which factors significantly contributed to the risk of graft failure and death with a functioning graft up to 10 years posttransplant.[77] We used the Kaplan-Meier method to analyze death censored GS in recipients. Univariate linear regression models were constructed with DGF, or one, five, and 10-year death censored graft survival as dependent variable and donor age as independent variable. In the model for DGF, the data were split into patients who received a DCD kidney and those who received a graft derived from donation after brain death (DBD), since DCD has a well documented independent effect on the incidence of DGF.[40,48]

We followed the approach outlined in figure 4a to simulate graft survival, as if an old-for-old allocation program had been employed in the time period studied. Old-for-old matching was performed following the Eurotransplant Senior Program (ESP) allocation rules: Donor and recipient age ≥65 years, only recipients with no prior transplants, recipient panel reactive antibodies (PRA) ≤5%, no human leukocyte antigen (HLA) matching, and a policy to keep cold ischemic time (CIT) relatively short. For our old-for-old simulation, CITs of 65+ grafts were artificially reduced by a factor 12/19, thus mimicking the effect observed in the ESP.[78] For each existing or newly matched donor kidney + recipient combination, a theoretical graft survival time was calculated. Based on the shape of the actual baseline survival data points underlying a Cox model for graft failure in our dataset, we estimated that the baseline survival function would follow an exponential course:

$$[1] \quad S_0(t) = a \cdot e^{-c \cdot t}$$

where t is time posttransplant. Values for *a* and *c* were derived by means of a least square fit to the baseline survival data points derived from this Cox model. Next, a survival function was obtained for each existing or newly matched combination:

$$[2] \quad S(t) = S_0(t)^{e^{\sum_1^i (b_i \cdot x_i)}}$$

where b_i is the *i*-th regression coefficient and x_i is the value of the *i*-th factor in the Cox model.[77] From equations [1] and [2], an equation for graft survival time (time-to-failure) of any donor kidney + recipient combination was derived:

$$[3] \quad T(s) = -\frac{1}{c \cdot \sum_1^i (b_i \cdot x_i)} \cdot \ln\left(\frac{s}{a^{\sum_1^i b_i \cdot x_i}}\right)$$

where *s* is a random number between 0 and 1 generated for each recipient, and *T* is the simulated time-to-failure for the graft.

Statistical analyses were conducted using SPSS and SigmaPlot software. Two-sided p-values <0.05 were considered to indicate statistical significance.

Donor demographics	Whole cohort	Only 65+ donors
Donor age[a] (yr)	39 (11–85)	67 (65–85)
Female donor (%)	41	55
DCD donor (%)	4	2
ECD donor (%)	15	100
Traumatic cause of death (%)	46	14
Donor history of hypertension (%)	21	46
Donor history of diabetes mellitus (%)	4	7
Recipient demographics		
Recipient age[a] (yr)	49 (0–90)	60 (6–90)
Female recipient (%)	39	38
Total time spent on the waiting list[a] (yr)	1.4 (0–22)	1.4 (0–16)
Previous transplants (% ≥1)	10	5
PRA level >5% (%)	19	13
Transplant demographics		
HLA mismatches (% of 0 mismatches)	16	7
Hypothermic machine perfusion (%)	15	24
Cold ischemic time[a] (h)	18 (0–78)	19 (0–67)

Table 1: Donor, recipient, and transplant demographics for the whole study cohort (n = 99,860 deceased donor kidney transplants between 1994 and 2006), and for all kidney transplants performed from deceased donors aged 65 years and older in this same cohort (n = 1,011).
[a] Median (range).

RESULTS

Demographics

Between January 1, 1994 and December 31, 2006, 99,860 deceased donor single-kidney transplants from donors aged ≥11 years were performed in the USA. Table 1 shows basic demographic statistics for the study population. Figure 1a shows that in young donors, the leading cause of death was trauma, whereas in older donors death following a cerebrovascular accident (CVA) was predominant. Between 1994 and 2006, the total number of kidney transplants per year from deceased donors increased by 39.5% (fig. 1b). This increase came primarily from donors above the age of 35, and therefore the relative share of older donor kidney transplants has risen during these 13 years. There was no relevant correlation between donor and recipient age in this dataset ($R^2 = 0.05$).

Table 2

Variable	Odds ratio / Hazard ratio (95% CI)[b]	P-value
Delayed graft function		
Donor age (yr)	1.02 (1.02–1.02)	<0.0005
DCD donor vs. DBD donor	3.01 (2.86–3.32)	<0.0005
ECD donor vs. non-ECD donor	0.99 (0.94–1.04)	0.6
Donor cause of death: CVA	1.02 (0.97–1.07)	0.4
Donor cause of death: trauma	0.87 (0.82–0.91)	<0.0005
Donor history of hypertension	1.33 (1.28–1.39)	<0.0005
Donor history of diabetes mellitus	0.99 (0.92–1.06)	0.8
Machine perfusion vs. static storage	0.53 (0.51–0.56)	<0.0005
Cold ischemic time (hrs)	1.04 (1.04–1.05)	<0.0005
Number of HLA mismatches	1.08 (1.07–1.09)	<0.0005
Recipient age (yr)	1.00 (1.00–1.00)	0.002
Total time spent on the waiting list (yr)	1.10 (1.09–1.11)	<0.0005
Most recent PRA level (%)	1.00 (1.00–1.00)	<0.0005
Number of previous kidney transplants	1.22 (1.16–1.28)	<0.0005
Graft loss within three months posttransplant (surrogate for primary non-function)		
Donor age (yr)	1.01 (1.01–1.01)	<0.0005
DCD donor vs. DBD donor	1.31 (1.13–1.53)	<0.0005
ECD donor vs. non-ECD donor	1.35 (1.22–1.48)	<0.0005
Donor cause of death: CVA	1.27 (1.14–1.41)	<0.0005
Donor cause of death: trauma	1.02 (0.92–1.13)	0.8
Donor history of hypertension	1.14 (1.05–1.23)	0.002
Donor history of diabetes mellitus	1.13 (0.99–1.30)	0.07
Machine perfusion vs. static storage	0.94 (0.87–1.03)	0.2

Table 2 Continued

Variable	Odds ratio / Hazard ratio (95% CI)[b]	P-value
Cold ischemic time (hrs)	1.02 (1.02–1.02)	<0.0005
Number of HLA mismatches	1.09 (1.07–1.11)	<0.0005
Recipient age (yr)	0.99 (0.99–0.99)	<0.0005
Total time spent on the waiting list (yr)	1.02 (1.01–1.04)	0.006
Most recent PRA level (%)	1.01 (1.00–1.01)	<0.0005
Number of previous kidney transplants	1.43 (1.32–1.55)	<0.0005
Graft failure within the first 10 years posttransplant[c]		
Donor age (yr)	1.01 (1.01–1.01)	<0.0005
DCD donor vs. DBD donor	0.89 (0.81–0.97)	0.009
ECD donor vs. non-ECD donor	1.21 (1.16–1.27)	<0.0005
Donor cause of death: CVA	1.05 (1.00–1.11)	0.04
Donor cause of death: trauma	0.99 (0.94–1.04)	0.7
Donor history of hypertension	1.08 (1.04–1.13)	<0.0005
Donor history of diabetes mellitus	1.23 (1.15–1.32)	<0.0005
Machine perfusion vs. static storage	1.09 (1.05–1.14)	<0.0005
Cold ischemic time (hrs)	1.00 (1.00–1.01)	0.001
Number of HLA mismatches	1.09 (1.08–1.10)	<0.0005
Recipient age (yr)	0.98 (0.98–0.98)	<0.0005
Total time spent on the waiting list (yr)	1.00 (0.99–1.00)	0.2
Most recent PRA level (%)	1.00 (1.00–1.01)	<0.0005
Number of previous kidney transplants	1.22 (1.17–1.27)	<0.0005
DGF vs. no DGF in recipient	2.22 (2.15–2.28)	<0.0005
Death with a functioning graft within the first 10 years posttransplant		
Donor age (yr)	1.00 (1.00–1.01)[d]	<0.0005
DCD donor vs. DBD donor	0.87 (0.76–0.98)	0.03
ECD donor vs. non-ECD donor	1.06 (0.99–1.13)	0.08
Donor cause of death: CVA	1.06 (1.00–1.14)	0.07
Donor cause of death: trauma	1.01 (0.95–1.08)	0.8
Donor history of hypertension	1.02 (0.97–1.08)	0.4
Donor history of diabetes mellitus	1.13 (1.03–1.24)	0.01
Machine perfusion vs. static storage	1.01 (0.95–1.07)	0.7
Cold ischemic time (hrs)	1.00 (1.00–1.00)	0.2
Number of HLA mismatches	1.00 (0.99–1.02)	0.5
Recipient age (yr)	1.05 (1.05–1.05)	<0.0005
Total time spent on the waiting list (yr)	1.02 (1.01–1.03)	0.004
Most recent PRA level (%)	1.00 (1.00–1.00)	0.002
Number of previous kidney transplants	1.10 (1.03–1.18)	0.007
DGF vs. no DGF in recipient	1.45 (1.39–1.51)	<0.0005

Table 2: Multivariate risk analysis[a] for delayed graft function, early graft loss, graft failure, and death with a functioning graft.

[a] Logistic regression models for delayed graft function and for graft loss within three months posttransplant, and Cox proportional hazards models for graft failure and for patient death with a functioning graft. For each covariate in the Cox models, the proportional hazards assumption was tested graphically with stratified survival plots for binary covariates, and with partial residual plots for non-binary covariates. We judged that the assumption was well met for all covariates in the models, except for traumatic cause of death in the Cox model for graft failure in the first 10 years posttransplant. However, since the effect of this covariate in the model was minimal and non-significant, it was not converted into a time-dependent covariate.

[b] Odds ratios apply to the logistic regression models and hazard ratios apply to the Cox proportional hazards models.

[c] Censored upon death with a functioning graft.

[d] Value in three decimal numbers: 1.004 (1.002–1.006)

Kidney discard

In figure 2a relative deceased donor kidney usage is plotted as the percentage of kidneys that were actually transplanted from donors recovered each year. 35+ Donor kidney usage decreased between 1994 and 2006, especially in kidneys derived from 50+ donors. Discard rates were higher for each subsequent donor age category, up to 36.9% and 66.9% for 50–64 year old and 65+ donor kidneys, respectively in 2006.

Risk factors for delayed graft function and early graft loss

In a binary logistic regression model, all included factors were significant independent determinants of the risk for DGF, except for ECD donor vs. non-ECD donor, CVA as cause of death in the donor, and donor history of diabetes mellitus (table 2). Donor age increased DGF risk with an OR of 1.02 ($p < 0.0005$), indicating that for each year increase in donor age, the relative risk for DGF in the recipient increased by 2%. Each additional year of donor age significantly increased the risk of early graft loss with an OR of 1.01 ($p < 0.0005$).

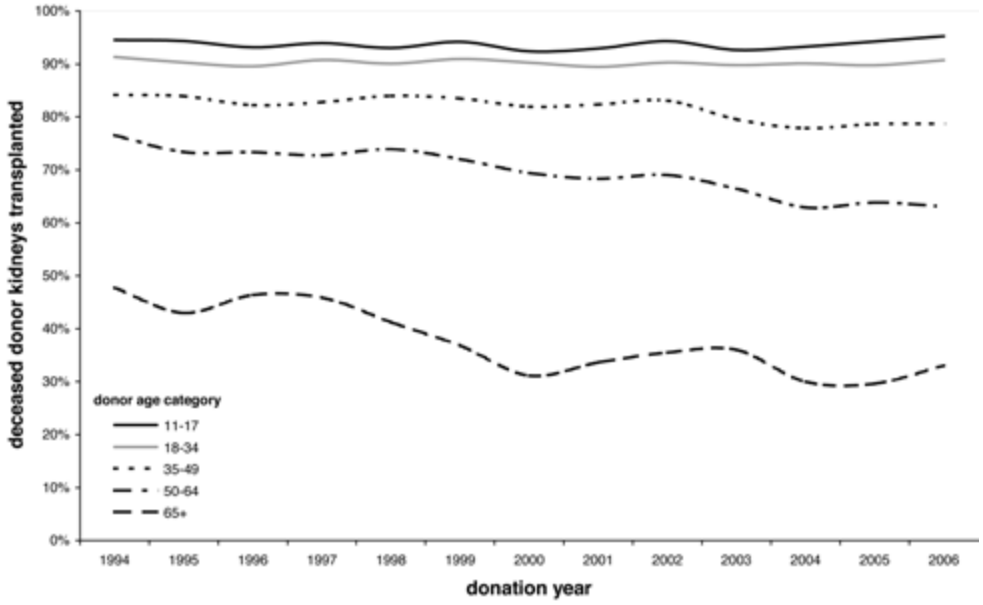
Risk factors for graft failure and death with a functioning graft

All factors included in the Cox model, except traumatic cause of death of the donor and the number of years the recipient spent on the waiting list, significantly influenced the risk of graft failure. Donor age increased the risk of graft failure with a HR of 1.01 ($p < 0.0005$) for each subsequent year of age. Each year increase in donor age was also associated with a significantly higher risk of recipient death with a functioning graft (OR 1.004, $p < 0.0005$).

Kaplan-Meier graft survival analysis

Figure 2b shows that for each subsequent donor age category above 11–34, graft survival up to 10 years was significantly lower (log rank test, $p < 0.0005$). Graft survival was as low as 39% at 10 years posttransplant for 65+ donor grafts, versus 70% for kidneys derived from donors aged 11–34 years.

a



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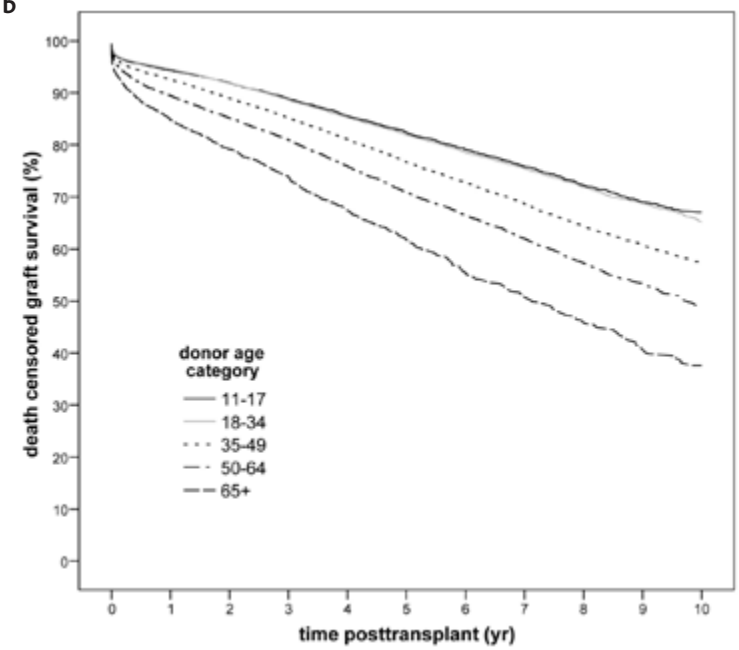


Figure 2: a) Relative deceased donor kidney usage: Percentage of kidneys actually transplanted from all donors recovered each year, per donor age category; **b)** Kaplan-Meier plots of 10-year death censored graft survival in recipients of deceased donor kidneys, stratified into five different donor age categories.

DGF risk as a function of donor age

In figure 3a the incidence of DGF is plotted with donor age as an independent variable, stratified into DBD and DCD donor kidneys. These univariate regression analyses show that the absolute risk of DGF increased by 0.35% and 0.37% with each year increase in donor age for DBD and DCD grafts, respectively ($p < 0.0005$). DGF risk in DCD kidneys derived from donors aged 11–65 years was 17–18% higher than in DBD grafts. Both effects were present for the whole spectrum of donor ages between 11 and 75 years ($R^2 = 0.90$ for DBD recipients and $R^2 = 0.30$ for DCD recipients).

Graft survival as a function of donor age

Figure 3b shows 1-, 5-, and 10-year death censored graft survival rates as a function of donor age, for three different cohorts within the study population. Quadratic univariate regression functions were fitted to these data points, which yielded three curves predicting death censored graft survival. For all three follow-up intervals, graft survival rates decreased when donor age increased. This effect was present for the whole range of donor ages between 11 and 75 years, and most pronounced with advanced donor age.

Simulation of old-for-old allocation

In order to give an impression of the simulation accuracy of our model, figure 4d shows 10-year death censored graft survival for the entire study population. One Kaplan-Meier curve is based on real survival data, while the other curve was simulated for the same cohort of recipients. Although the model failed to accurately predict graft survival in the first year, differences between the real and the simulated Kaplan-Meier curve were only minimal from 1 year posttransplant onwards. At 10 years after transplantation both curves virtually overlapped.

In our analysis, donor age was a significant independent risk factor for death with a functioning graft in a Cox model for this outcome (Table 2), but there was no significant interaction between donor age and recipient age in the model ($p = 0.5$), implying that an old patient receiving an old kidney is not more at risk for death with a functioning graft than a younger patient who receives an old kidney. Figures 4b and 4c show the results of the old-for-old allocation simulation outlined in figure 4a. 65+ Grafts that are normally predominantly allocated to recipients aged <65 years (old-to-young) showed a drop in 10-year graft survival when artificially re-allocated to recipients aged ≥65 years (old-to-old) (20.9% => 12.9%; $p < 0.0005$). When cases were censored upon death with a functioning graft (fig. 4c), this difference disappeared (39.7% => 38.9%; $p = 0.9$). Younger-than-65 donor grafts which were previously allocated to recipients of 65 years and older (yound-to-old) may have a better 10-year graft survival if these kidneys are allocated to recipients under 65 years (young-to-young), although the difference observed did not reach statistical significance (19.4% => 26.2%; $p = 0.4$). When censored upon death with a functioning graft, no such improvement could be observed anymore (56.1% => 53.5%; $p = 0.05$).

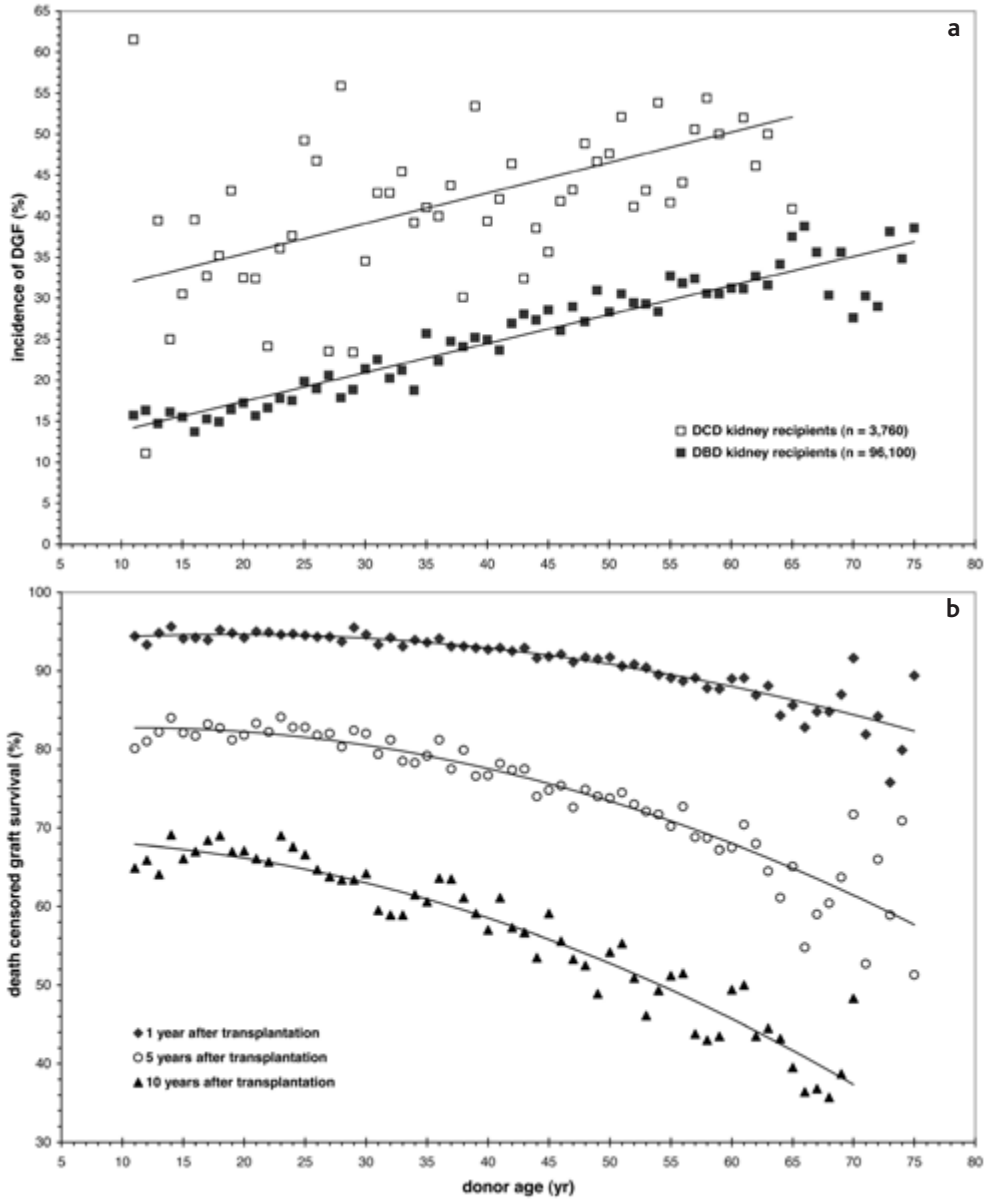


Figure 3: a) The incidence of DGF per year of donor age, with fitted regression lines predicting DGF risk. Linear regression equations obtained from these data are as follows: [%DGF in DBD recipients] = 0.35 x [donor age] + 10; [%DGF in DCD recipients] = 0.37 x [donor age] + 27. In the univariate regression analysis for DGF, a quadratic (or even higher order) fit did not improve the R^2 to such an extent that it would be relevant to incorporate this more complex approximation; **b)** 1-, 5-, and 10-year graft survival rates per year of donor age, with fitted quadratic regression curves predicting graft survival. The equations obtained for these curves are as follows: [1-year GS] = -0.0039 x [donor age]^2 + 0.15 x [donor age] + 93 (R^2 = 0.81, $p < 0.0005$); [5-year GS] = -0.0061 x [donor age]^2 + 0.13 x [donor age] + 82 (R^2 = 0.86, $p < 0.0005$); [10-year GS] = -0.0065 x [donor age]^2 + 0.0079 x [donor age] + 69 (R^2 = 0.91, $p < 0.0005$). For this analysis, a quadratic fit yielded much better R^2 values than a linear fit.

Recipients aged 65 years and older who had previously received a graft derived from a donor under the age of 65, had a significantly worse 10-year graft survival in the hypothetical new situation where they would have received a 65+ graft (non-death censored: 19.4% => 12.9%; p < 0.0005, death censored: 56.1% => 38.9%; p < 0.0005). Conversely, recipients under the age of 65 who had originally received a 65+ graft, would now receive a kidney with a significantly improved 10-year graft survival (non-death censored: 20.9% => 26.2%; p = 0.001, death censored: 39.7% => 53.5%; p < 0.0005). As the curves in figures 4b and 4c demonstrate, the disadvantage for the former recipients would approximately equal the benefit in the latter group. For reference, figures 4b and 4c also show a real, non-simulated survival curve of those 1,011 65+ donor kidneys that were actually transplanted to non-immunized 65+ recipients in the study cohort.

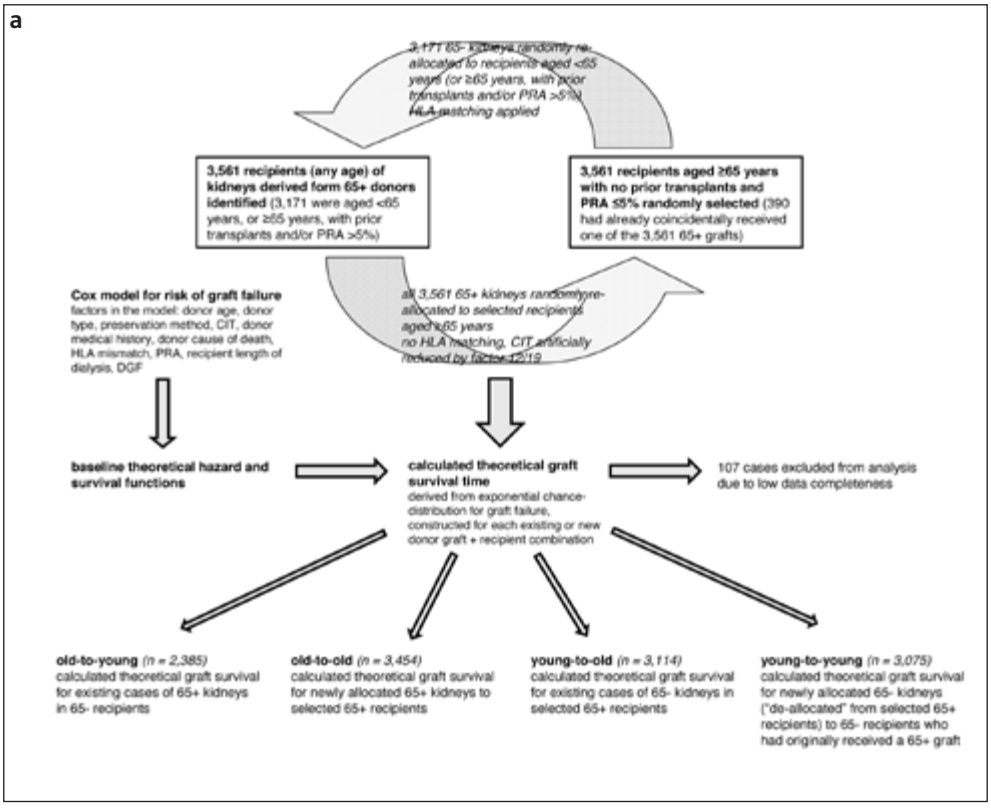


Figure 4: a) Schematic overview of old-for-old simulation methodology.

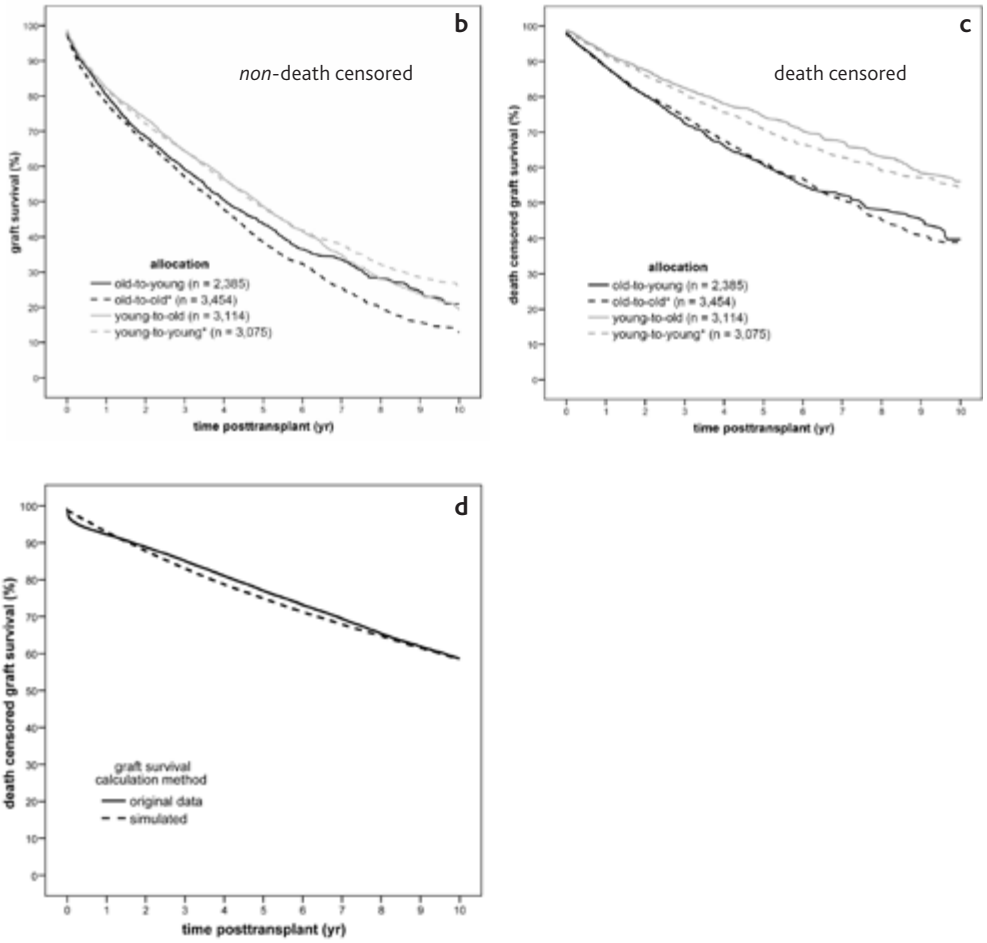


Figure 4: b) 10-year *non*-death censored graft survival after simulation; **c)** 10-year death censored graft survival after simulation; **d)** Impression of model simulation accuracy: Overall 10-year death censored graft survival for the whole study cohort, based on real graft survival data, and on simulated values for the same cohort of recipients (n = 99,860). The real, non-simulated old-for-old survival curves in figures 4b and 4c should be regarded as reference only and cannot be compared with the simulated old-for-old curves. The reason is that in those 1,011 real transplants HLA matching was performed, whereas in the simulation kidneys were not matched for HLA and CITs were artificially reduced by a factor 12/19. * = *Newly* matched donor graft + recipient combinations.

DISCUSSION

In this retrospective OPTN database analysis, we have shown that donor age has an important impact on short- and long-term outcome after deceased donor kidney transplantation. This is true not only for selected categories, but for the whole range of donors aged 11 years and older. From 1994 through 2006, the relative share of older donor grafts in the kidney transplant pool has increased. Older donor kidney discard rates in the USA are known to be higher than in other parts of the world and there is much debate why this discrepancy exists.[79] Donor biopsies may have contributed to these statistics: Cecka and colleagues showed that in the USA the percentage of donor kidneys biopsied increases with donor age, thus giving recipient centers an additional diagnostic tool to select kidney grafts derived from older donors.[79] It is plausible that transplant centers are hesitant to accept kidneys with a documented high percentage of glomerulosclerosis, even if other organ quality measures are favorable and the centers would have accepted the kidney without a biopsy. Apart from biopsy interpretation, donor age itself may also be an important motivation for not accepting kidneys from older donors.[80] Irrespective of the reason for kidney discard, the impact of advanced donor age on outcome may be underestimated due to the fact that more stringent qualitative selection criteria are applied to older donor grafts, compared to younger donor kidneys. It should be kept in mind that our results represent the effect of donor age in the context of current clinical transplantation practice in the USA, and in the presence of a high kidney discard rate for donors of advanced age.

An important limitation of our study is that we had to use early graft loss as a surrogate marker for primary non-function, and that DGF was the only available indicator for early renal function. Serum creatinine or creatinine clearance values would have offered a more detailed tool to assess graft function, but these were not available in the database at standardized time points after transplantation.

In the context of relevant covariates, donor age proved to be an independent predictor of DGF risk, early graft loss, and graft failure within 10 years posttransplant. Although the odds and hazard ratios seem rather low at first sight, it should be noted that donor age was included in the model as a continuous variable, in contrast to most analyses where donor age is a categorical variable with only a few possible alternatives. The Kaplan-Meier analyses show that for each subsequent donor age category above 11–34 years, death censored GS up to 10 years was significantly lower. The quadratic regression models present a more detailed analysis, which reveals that there is a weak negative effect of donor age on GS for donor ages ≤34. This effect becomes increasingly stronger from this age onwards. Not surprisingly, the incidence of DGF was higher in DCD kidney recipients. Overall, the 17–18% higher rate versus DBD graft recipients is well in line with findings of other studies.[40,67,81-83] The higher variance in the DCD data can be explained by the smaller number of available cases per single year of donor age (~60 DCD recipients versus ~1500 DBD recipients). These univariate data show that donor age accurately predicts DGF risk, not only in the upper extremes, but for the whole range of deceased donor ages between 11 and 75 years.

Advanced recipient age does not appear to be a relevant risk factor for an inferior short- or long-term posttransplant graft outcome, when death with a functioning graft is not considered a failure. The multivariate Cox model even points at a graft survival benefit when recipient age increases. However, this observation is most likely to be an artifact associated with censoring cases upon death with a functioning graft in combination with the long survival period studied by the model.

If advanced recipient age does not negatively contribute to death censored posttransplant graft outcome, old-for-old allocation could become interesting. Between 1994 and 2006 some organ procurement organizations in the USA have practiced old-for-old kidney allocation.[84] When we correlated donor age with recipient age, however, we found that in the time period studied such policies did not exist on a large scale. An important rationale for senior recipient exchange policies is that a transplanted kidney which outlives its recipient can be considered a success. Theoretically, it would be expected that the disadvantage of receiving an older kidney is less severe in aged recipients, since they have a higher chance of dying before the relatively short lifespan of their graft is over. Conversely, kidney grafts derived from younger donors would be expected to have a higher yield in younger recipients, as these patients would make more use of the longer lifespan that a young graft has to offer. Although both effects mentioned above were observed in our simulation, the net effect of implementing an old-for-old allocation program in terms of total functional graft time gained or lost would most likely be close to zero. Our analysis did show that 65+ grafts will function equally well in non-HLA matched older recipients, compared to their performance in HLA matched younger patients. The simulated data suggest that it could be safe to sacrifice HLA matching for obtaining shorter CITs and – perhaps even more important – reducing waiting times of selected senior transplant candidates. Results of the ESP show that the system also significantly reduced discard rates of aged donor kidneys,[85] but it is difficult to predict whether the same would happen in the USA, since the exact reasons for current high kidney discard rates remain elusive. As a side effect, allocation of kidney grafts derived from relatively young donors to relatively young recipients may even further improve outcomes for this group. We found that re-transplantation is associated with a marked increase in the risk of graft failure. This may be a good reason to give longer-lasting kidneys to longer-living patients, in order to keep the number of re-transplants as low as possible for recipients in this group. Our calculations also showed that advanced donor age puts a recipient more at risk for death with a functioning graft. Although the magnitude of this effect does not differ between old and young recipients, an old-for-old policy might imply that the average patient survival of older recipients decreases, whereas patient survival will increase with the same amount in younger recipients. Such potential shifting of life years from one group to another is likely to cause serious ethical dilemmas for policy makers. It is difficult to predict whether a shorter time on dialysis for older patients will balance or even outweigh this survival disadvantage, but reports from existing old-for-old programs suggest that the algorithm can be implemented without compromising graft and patient survival.[76,78,84-89] Nevertheless, only 3% of all

kidney transplants in our study's cohort came from donors aged 65 years and older. If a senior recipient allocation program is to make any difference on overall renal transplant outcome, older kidney utilization rates in the USA would probably have to become substantially higher. Presently, introducing such a new algorithm seems to be much effort for only a very small percentage of the transplant population, with a net benefit which is likely to be nearly zero.

A possible limitation of our old-for-old analysis is that part of it is inevitably bas ed on theoretical assumptions about the survival conduct of a graft in its recipient. However, all calculations are also based on real donor kidneys and real recipients in the OPTN database, with many of their relevant characteristics taken into account by means of a Cox model. Formal mathematical sensitivity analyses are beyond the scope of this paper, but our model did replicate the overall real graft survival curve with a high accuracy. This suggests that the simulated survival curves are likely to reliably predict clinical reality. Another potential limitation is our assumption that old-for-old allocation would decrease CIT for 65+ kidneys in the USA with the same amount as it did in Eurotransplant. It remains to be seen whether this is true, since the current American ECD program already accounts for a relatively short CIT when donor age is above 60.

In summary, our results show that deceased donor age is a strong predictor of inferior short- and long-term outcome after kidney transplantation for the whole range of donor ages from 11 years and above. When it is taken into account that older donor grafts are subjected to an exceptionally strict quality selection in the USA,[79] the real biological adverse effect of donor age on outcome may even be more pronounced. As a result of this selection, kidney graft utilization rates from old donors are very low, with discard rates up to 66.9% for 65+ donors. Even with such high kidney discard in older donors, the risk of DGF, early and late graft failure increases for each subsequent year of donor age. Provided that the average CIT of 65+ kidneys would decrease with a few hours by abandoning HLA matching, broad implementation of old-for-old kidney allocation in the USA is likely to be safe and could be a tool to reduce waiting time for older patients. However, our simulation suggests that it will not necessarily improve the overall outcome after kidney transplantation.

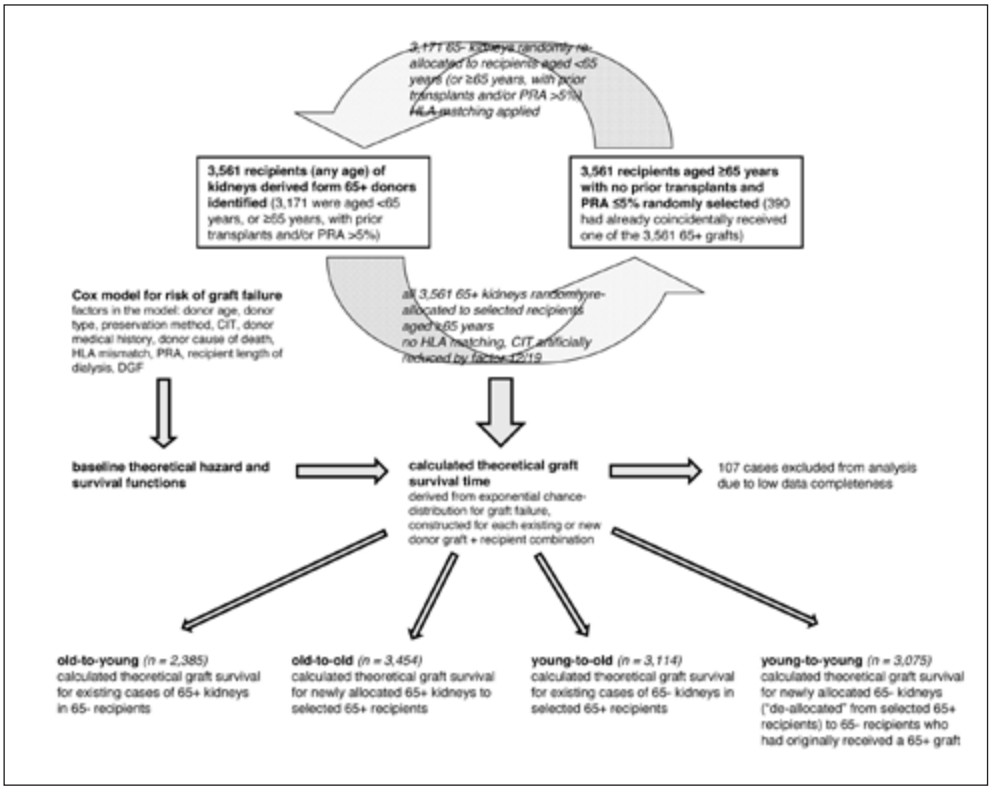
OFFICIAL STATEMENT OF ACKNOWLEDGEMENT

This work was supported in part by Health Resources and Services Administration contract 231–00–0115. The content is the responsibility of the authors alone and does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

SUPPLEMENTARY APPENDIX

Old-for-old simulation methodology

We followed the approach outlined in the figure below (Fig. 4a in the article) to simulate graft survival, as if an old-for-old allocation program had been employed in the time period studied. Old-for-old matching was performed following the Eurotransplant Senior Program (ESP) allocation rules: Donor and recipient age ≥65 years, only recipients with no prior transplants, recipient panel reactive antibodies (PRA) ≤5%, no human leukocyte antigen (HLA) matching, and a policy to keep cold ischemic time (CIT) relatively short. For our old-for-old simulation, CITs of 65+ grafts were artificially reduced by a factor 12/19, thus mimicking the effect observed in the ESP:



For each existing or newly matched donor kidney + recipient combination, a theoretical graft survival time was calculated, following the methodology outlined below.

In Cox proportional hazards analysis, the model is given by:

$$[1] \quad H(t) = H_0(t) \cdot e^{\sum_1^i (b_i \cdot x_i)}$$

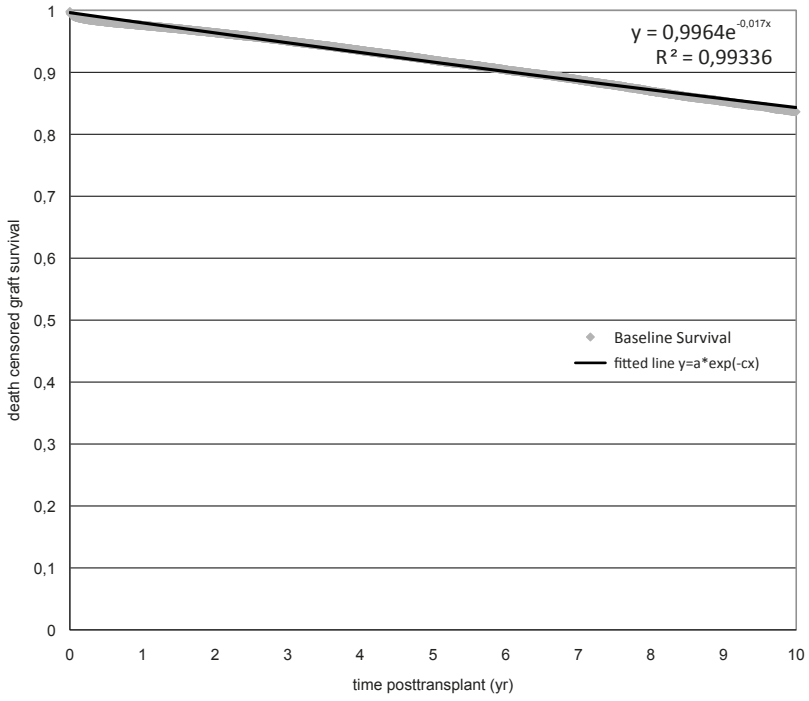
where $H(t)$ is the hazard function for a given set of covariates with their respective values, $H_0(t)$ is the baseline hazard function (the hazard function when all covariates are at their baseline value), b_i is the i-th regression coefficient, and x_i is the value of the i-th covariate in the Cox model. The hazard function can be converted into a survival function:

[2] $$S(t) = e^{-H(t)}$$

This also implies that the baseline survival function (the survival function when all covariates in the Cox model are at their baseline value) will be:

[3] $$S_0(t) = e^{-H_0(t)}$$

Using SPSS, we derived the actual baseline survival function data points underlying a Cox model for graft failure in our dataset. These data points were plotted in a graph:



Based on the shape of this graph, and on established knowledge about survival analysis in organ transplantation, we estimated that the baseline survival function would follow an exponential course:

[4] $$S_0(t) = a \cdot e^{-c \cdot t}$$

where t is time posttransplant. Values for *a* and *c* were derived by means of a least square fit to the baseline survival data points derived from this Cox model (black fitted line in figure above, with values for *a* and *c* shown in the fitted formula, upper right corner). Next, a survival function was obtained for each existing or newly matched combination by combining equations [1], [2], and [3]:

[5] $$S(t) = S_0(t)^{e^{\sum_1^i (b_i \cdot x_i)}}$$

where b_i is the *i*-th regression coefficient and x_i is the value of the *i*-th factor in the Cox model. However, to construct a Kaplan-Meier graft survival curve for the whole group of newly matched pairs, we needed a distinct time-to-failure for each new case, not a survival function. By combining equations [4] and [5], an equation for graft survival time (time-to-failure) of any donor kidney + recipient combination was derived:

[6] $$T(s) = -\frac{1}{c \cdot e^{\sum_1^i (b_i \cdot x_i)}} \cdot \ln\left(\frac{s}{a^{\sum_1^i b_i \cdot x_i}}\right)$$

where *s* is a random number between 0 and 1 generated for each recipient, and *T* is the simulated time-to-failure for the graft. The random number *s* can be understood as follows: By converting equations [4] and [5] from *S(t)* into *T(s)* (by simple algebraic inversion), equation [6] was obtained. Graphically, this can be visualized as switching both axes of the survival plot. The number *s* would actually represent the survival probability (possible range 0-1) for this particular new case at a given time posttransplant. Since time is now a function of the survival probability, this survival probability *s* needs to be filled in into equation [6] to obtain a time-to-failure for this case. If a probability needs to be filled in, there is no reason why any particular value between 0 and 1 would be favoured more than another. Therefore, for each simulated new case, we generated a random number between 0 and 1 for *s* and filled in this number into equation [6]. Doing so yielded a simulated time-to-failure for this particular case. The basis for this calculation is pure chance, but then this chance is "weighed" by the shape of the survival curve for this unique case, defined by the weight of all individual risk factors that apply for this newly matched donor kidney and recipient pair.

Using the calculated time-to-failure value of each new case, the simulated Kaplan-Meier plots (fig. 4b and 4c in the paper) were produced.

Chapter 4

The effect of normothermic recirculation before cold preservation on posttransplant injury of ischemically damaged donor kidneys

Article in press, Transplant International 2011

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ABSTRACT

Kidneys recovered from donation after cardiac death (DCD) are increasingly used to enlarge the deceased donor pool. Such renal grafts, especially those derived from uncontrolled DCD, have inevitably sustained profound warm ischemic injury, which compromises posttransplant function. Normothermic recirculation (NR) of the deceased donor's body before organ cooling could be an interesting approach to mitigate the detrimental effect of warm ischemia. To date, however, there is no evidence coming from preclinical studies to support the principle of NR in kidney transplantation. In this study, we subjected 48 Lewis rat kidneys to 15 or 30 min of warm ischemia, and subsequently 0, 1, or 2 h of NR. After 24 h cold storage kidneys were transplanted into a recipient animal and 24 h later we measured the percentage of cortical necrosis, and determined gene expression of heme oxigenase-1, heat shock protein-70, transforming growth factor-β, kidney injury molecule-1, interleukin-6, hypoxia inducible factor-1α, monocyte chemoattractant protein-1, and α-smooth muscle actin in kidney tissue. We found that NR had no significant influence on any of these markers. Therefore, we conclude that this preclinical study by no means supports the presumed beneficial effect of NR on kidneys that have been severely damaged by warm ischemia.

INTRODUCTION

To partially resolve the persistent donor organ shortage, kidneys recovered from donation after cardiac death (DCD) are increasingly used to enlarge the deceased donor pool. Compared to renal grafts recovered from donors after brain death, DCD kidneys have by definition sustained additional injury due to warm ischemia (WI) between cardiocirculatory arrest and cold organ perfusion. Although the duration of WI varies among the different types of DCD donors, recipients of such kidneys are known to have a substantially increased risk of delayed graft function and primary non-function, especially when WI has been very profound such as in uncontrolled DCD.[90]

Most established organ preservation protocols are based on rapid cooling immediately after cardiac arrest, followed by organ procurement and either static cold storage or hypothermic machine perfusion of the kidney graft.[91] To mitigate the detrimental effect of warm ischemia, some studies have suggested the use of normothermic recirculation (NR) before organ cooling is instituted. NR is an early organ preservation strategy, in which the deceased donor's body is artificially recirculated with warm oxygenized blood quickly after the declaration of cardiocirculatory death, for a limited period of time such as one or two hours. NR is typically administered through an extracorporeal membrane oxygenator, connected to a closed circuit with cannulae in the femoral vessels of the deceased donor.[92] A few studies have reported beneficial effects of this strategy on posttransplant graft function and survival. Most of these reports focus on NR prior to DCD liver transplantation.[39,92,93] So far, only one published clinical study presented results of NR in renal transplantation.[39,94] The authors reported a significant reduction of delayed graft function and an improved graft survival after transplantation when NR was compared with a protocol in which organs were immediately cooled. To our knowledge, the *Hospital Clínic* in Barcelona, Spain – the group that published these data – is the only center worldwide with a clinical NR protocol for potential uncontrolled (Maastricht category I and II)[13] DCD donors.

Before a novel preservation strategy such as NR can be widely implemented in human renal transplantation practice, more basic evidence is needed to quantify the magnitude of its presumed effect and to unravel the mechanism through which NR could be beneficial to a DCD kidney graft. To date, there is no evidence coming from preclinical studies to support the principle of NR in kidney transplantation. We have conducted an animal study to investigate the potential of NR to reduce WI injury in a standardized renal transplantation model. Aim of the present study was to determine whether NR can reduce the amount of tubular necrosis after transplantation, and whether NR influences the expression of genes that are involved in renal damage, inflammation, interstitial fibrosis formation, cytoprotection, and tissue regeneration in kidneys that have sustained severe warm ischemic injury in the donor.

METHODS

Animals and housing

Ninety-six adult male Lewis rats weighing 250–300g, obtained from Harlan (Zeist, The Netherlands) were used as kidney donors (n=48) and recipients (n=48). Before surgery, animals were kept in standard polycarbonate housing (model 1354F, Tecniplast, Buguggiate, Italy), with a maximum of four animals together in one cage. After surgery, recipient animals were housed individually in the aforementioned polycarbonate housing. Throughout the experiment, all animals were allowed free access to a standard laboratory animal diet and acidified tap water. All experimental procedures were approved by the animal experiment committee of the University of Groningen, and the principles of laboratory animal care (NIH publication no. 85-23, revised 1985), as well as regulations imposed by the Dutch law on animal experiments were followed.

Experimental design

We employed a syngeneic Lewis to Lewis rat renal transplant model with orthotopic transplantation of the left donor kidney, leaving the recipient's native right kidney in situ. Renal grafts in six experimental groups (eight transplants per group) received either 15 or 30 min of WI, followed by either no NR, 1 h NR, or 2 h NR, and subsequently 24 h cold storage (CS) preservation and transplantation into a recipient animal. Recipient animals were sacrificed exactly 24 hours posttransplant. Experimental groups were as follows:

Group 1: 15 min WI – no NR – 24 h CS – transplantation – 24 h reperfusion in the recipient
Group 2: 30 min WI – no NR – 24 h CS – transplantation – 24 h reperfusion in the recipient
Group 3: 15 min WI – 1 h NR – 24 h CS – transplantation – 24 h reperfusion in the recipient
Group 4: 30 min WI – 1 h NR – 24 h CS – transplantation – 24 h reperfusion in the recipient
Group 5: 15 min WI – 2 h NR – 24 h CS – transplantation – 24 h reperfusion in the recipient
Group 6: 30 min WI – 2 h NR – 24 h CS – transplantation – 24 h reperfusion in the recipient

We chose 15 and 30 min for the duration of WI time, since we had previously demonstrated that the combination of 15 min WI and 24 h CS results in a seriously damaged kidney graft, leading to delayed graft function after transplantation.[95] Since NR is most interesting in the uncontrolled DCD (Maastricht categories I and II) setting which leads to severely damaged kidneys, we deliberately chose not to test the method on kidneys that have sustained only mild ischemic injury. We added the duration of 30 min WI to provide for an even heavier variant of this DCD animal model. We chose 1 h and 2 h for the duration of NR, as these seem realistic times to apply NR in the human setting, which are also comparable to the time periods that the Barcelona group reports for NR in their center.

Donor operation and organ preservation

After induction of inhalation anesthesia with 5% isoflurane/oxygen, donor animals received 250 IU heparin via the penile vein. Through a midline incision, the left kidney, both renal vessels, and the ureter were isolated. The left renal artery and vein were subsequently clamped for 15 min or 30 min to induce WI. In stated experimental groups NR was induced by removal of the clamps and reperfusion of the left kidney for 1 or 2 h. Next, a ligature was placed around the aorta, superior to the right renal artery, to prevent flushing of the liver and intestine. The inferior caval vein was cut and both kidneys were flushed by inserting a 20G needle into the aortic bifurcation and infusing 10 ml of 0.9% NaCl at 37 °C, directly followed by 10 ml of University of Wisconsin (UW) organ preservation solution at 4 °C. Glutathione (0.922 mg/ml) was freshly added to the UW solution. Immediately upon flushing, the left kidney was removed. Donor kidneys were preserved during exactly 24 h by means of static CS at 0–4 °C, submerged into 25 ml of UW solution with added glutathione.

Recipient operation

After induction with 5% isoflurane/oxygen, maintenance inhalation anaesthesia with 3% isoflurane/oxygen was used. Orthotopic kidney transplantation was performed on the left side: First, the native left kidney was removed after clamping both renal blood vessels. The graft renal artery was anastomosed end-to-end to the recipient's renal artery using eight interrupted Dafilon® 10-0 (B.Braun) non-absorbable sutures, and the graft renal vein was anastomosed to the recipient's renal vein with a running suture of the same material. Vascular anastomosis time was standardized to exactly 25 min for each procedure. The graft ureter was anastomosed end-to-end to the recipient ureter using four interrupted sutures. The abdominal fascia and skin were closed in layers with two separate absorbable Safil® 4-0 (B.Braun) running sutures. Analgesia was managed subcutaneously with buprenorfine: Animals received 0.01 mg/kg during surgery, 0.04 mg/kg immediately after transplantation, and 0.05 mg/kg 10-12 hours post surgery. An electric warming blanket was placed under the cage floor to prevent hypothermia in the first hours after surgery. At exactly 24 h posttransplant, recipient animals were sacrified by exsanguination under anesthesia.

Sample collection and analysis

At termination, the donor kidney was collected and one tissue sample was fixed in 4% formalin for histological examination. Another tissue sample was immediately snap frozen in liquid nitrogen. Histological slices were stained by the periodic acid-Schiff (PAS) method and were quantitatively assessed for cortical necrosis. Digital images of each slice were taken and Aperio ImageScope software was used to calculate the percentage cortical necrosis as the quotient of the necrotic cortical area and the total cortical area. Figure 1 shows a representative example of the scoring method.

Real-time quantitative RT-PCR (qPCR) analysis of heme oxigenase-1 (HO-1), heat shock protein-70 (HSP-70), transforming growth factor-β (TGF-β), kidney injury molecule-1 (KIM-1),

interleukin-6 (IL-6), hypoxia inducible factor-1α (HIF-1α), monocyte chemoattractant protein-1 (MCP-1), and α-smooth muscle actin (α-SMA) gene expression was performed to detect cytoprotection (HO-1 and HSP-70), tissue regeneration (TGF-β), renal tubular injury (KIM-1), aspecific inflammation (IL-6, HIF-1α, and MCP-1), and early signs of interstitial fibrosis (α-SMA) 24 h after transplantation. Amplification primers (Table 1) were designed with Primer Express software (Applied Biosystems, Foster City, CA, USA) and validated in a 6-step twofold dilution series. RNA was extracted from snap frozen tissue using TRIzol (Invitrogen, Breda, the Netherlands). Total RNA was treated with DNase I, Amp Grade (Invitrogen). cDNA synthesis was performed from 1 µg total RNA using T_{11}VN oligos and M-MLV Reverse Transcriptase, according to supplier's protocol (Invitrogen). Amplification and detection were performed with the ABI Prism 7900-HT Sequence Detection System (Applied Biosystems) using emission from SYBR Green (SYBR Green master mix, Applied Biosystems). All assays were performed in triplicate. After an initial activation step at 50 °C for 2 min and a hot start at 95 °C for 10 min, qPCR cycles consisted of 40 cycles of 95 °C for 15 sec and 60 °C for 60 sec. Gene expression was normalized with the mean of β-actin mRNA content and calculated relative to healthy controls. Results were expressed as $2^{-\Delta C_T}$ (C_T threshold cycle).

Gene	Forward primer	Reverse primer
β-actin	5'–GGAAATCGTGCGTGACATTAAA–3'	5'–GCGGCAGTGGCCATCTC–3'
HO-1	5'–CTCGCATGAACACTCTGGAGAT–3'	5'–GCAGGAAGGCGGTCTTAGC–3'
HSP-70	5'–GGTTGCATGTTCTTTGCGTTTA–3'	5'–GGTGGCAGTGCTGAGGTGTT–3'
TGF-β	5'–GCTCTTGTGACAGCAAAGATAATGTAC–3'	5'–CCTCGACGTTTGGGACTGAT–3'
KIM-1	5'–AGAGAGAGCAGGACACAGGCTTT–3'	5'–ACCCGTGGTAGTCCCAAACA–3'
IL-6	5'–CCAACTTCCAATGCTCTCCTAATG–3'	5'–TTCAAGTGCTTTCAAGAGTTGGAT–3'
HIF-1α	5'–GAACATGATGGCTCCCTTTTTC–3'	5'–CCTGGTTGCTGCAGTAACGTT–3'
MCP-1	5'–CTTTGAATGTGAACTTGACCCATAA–3'	5'–ACAGAAGTGCTTGAGGTGGTTGT–3'
α-SMA	5'–GAGAAAATGACCCAGATTATGTTTGA–3'	5'–GGACAGCACAGCCTGAATAGC–3'

Table 1: Primers used for qPCR analyses.

Statistics

To minimize the number of animals required per group, a 2x3 between-subjects factorial design was constructed in which the levels of two independent variables (WI time 15 or 30 min, and NR time 0, 1, or 2 h) were varied among the six groups. Using Mead's formula for sample size estimation in factorial designs, we calculated that a minimum of five animals per group would be required to obtain adequate statistical power. Some interaction between WI time and NR time could be assumed, and variances could be different among the six groups. In addition, prior experience showed that in 15-20% of the transplants a technical complication would occur. Therefore, we determined that the initial number of animals per group should be

eight. Statistical analyses were performed with SPSS software, version 18. One-way ANOVAs were performed which tested whether the dimensions WI time and NR time significantly influenced each of the nine dependent variables (cortical necrosis, and the expression of eight genes) after transplantation, and whether there was any significant interaction between WI time and NR time. In case a significant effect of NR time was found for a certain dependent variable, we used Turkey's post-hoc test to determine between which of the three levels of NR time the significant difference existed. Since none of the independent variables were normally distributed, all values were transformed to ranks before being entered into the analyses. Two-sided p-values below 0.05 were considered to indicate statistical significance.

Dependent variable	P-value for WI	P-value for NR
Cortical necrosis	0.014	0.34
HO-1	0.36	0.014
HSP-70	0.44	1.00
TGF-β	0.18	0.62
KIM-1	0.10	0.17
IL-6	0.13	0.84
HIF-1α	0.10	0.60
MCP-1	0.42	0.18
α-SMA	0.20	0.48

Table 2: P-values resulting from the one-way ANOVAs which tested whether either warm ischemic time, or normothermic recirculation time significantly influenced each dependent variable.

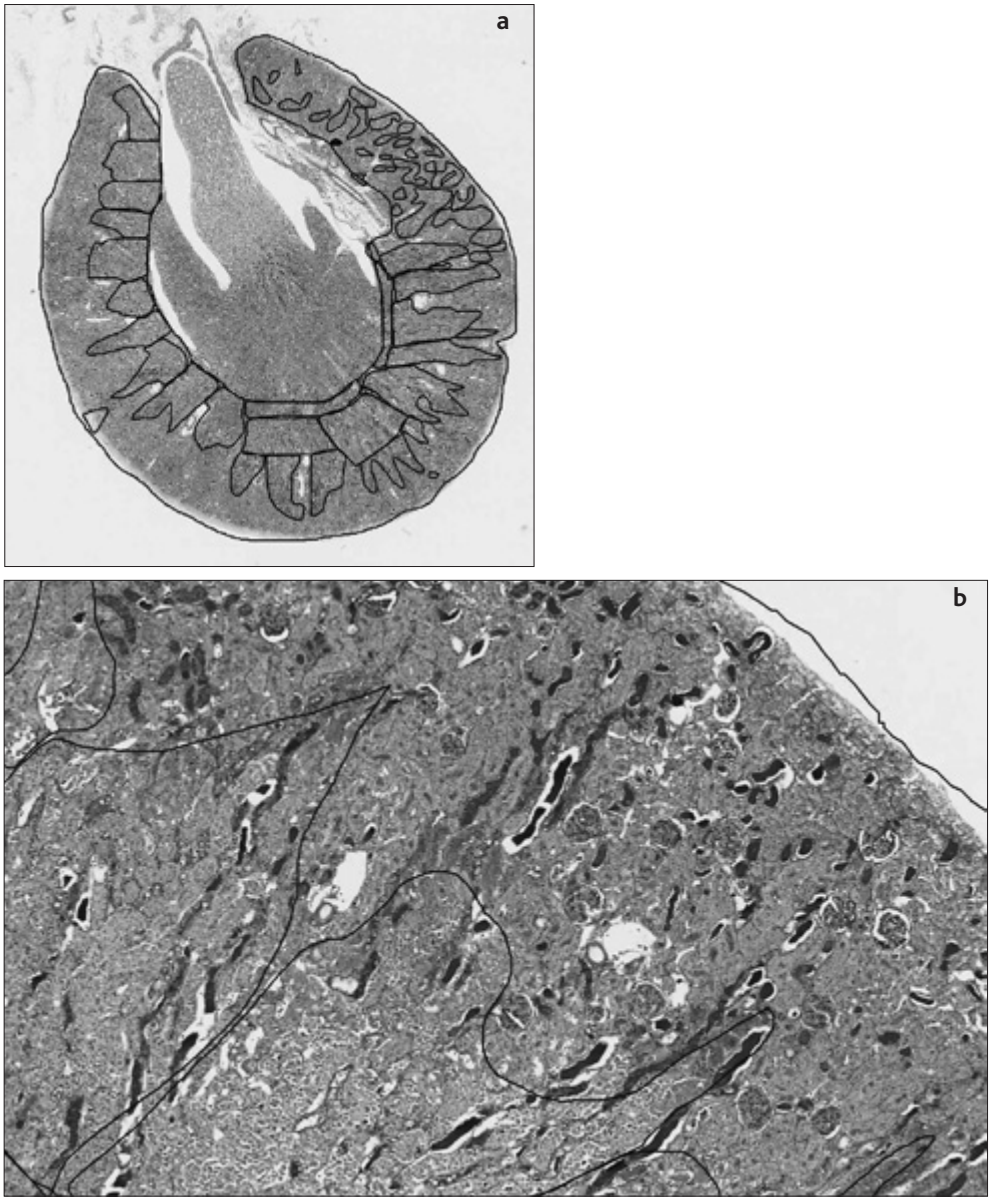


Figure 1: Representative example of the cortical necrosis scoring method. Histological slices were stained by the periodic acid-Schiff (PAS) method and were quantitatively assessed. Digital images of each slice were taken and Aperio ImageScope software was used to calculate the percentage cortical necrosis as the quotient of the necrotic cortical area and the total cortical area. Overview of a kidney at 10x magnification (**a**), and a more detailed view at 50x magnification (**b**). The total cortical area and the necrotic sections are encircled with black lines. In panel (**b**), the necrotic area on the lower left hand side is bounded by a black line, on the other side of which vital cortical renal tissue can be seen.

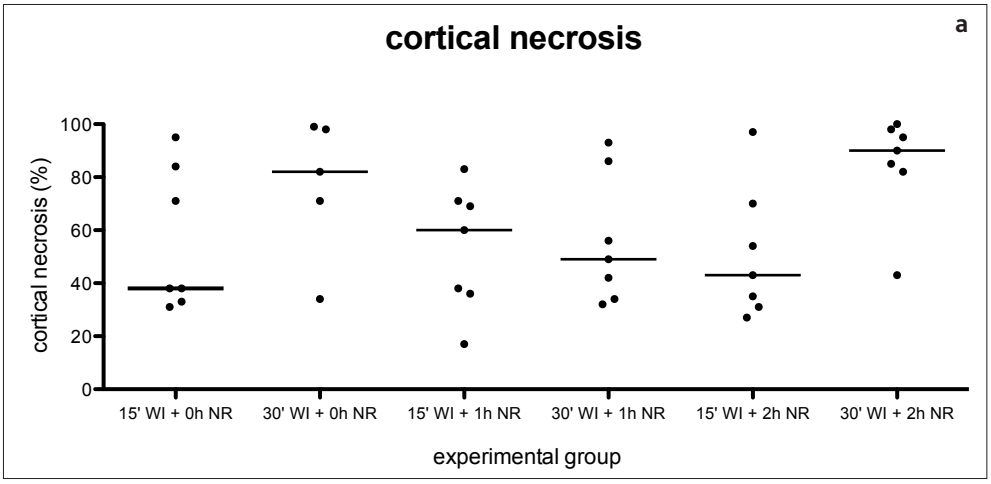
RESULTS

In nine out of 48 transplants (19%), technical complications occurred, which were mostly related to inadequate vascular flushout in the donor and/or leakage or occlusion of the vascular anastomosis in the recipient. These nine cases were excluded from further analysis. Exclusions led to a median of seven animals per experimental group. In the remainder of procedures, all recipient animals survived until sacrifice at 24 h after transplantation.

Cortical necrosis

Figure 2a shows a plot of the percentage cortical necrosis for each individual transplant, categorized per experimental group. In all groups, we found profound cortical necrosis 24 h after transplantation with an overall median of 65% (interquartile range 37–86%) of the total renal cortical area. In kidneys that had sustained 15 min of WI, the median cortical necrotic area was 43% (interquartile range 34–71%), whereas renal grafts with 30 min of WI in the donor had a significantly higher median cortical necrotic area of 82% (interquartile range 43–95%; P=0.01). In contrast, NR did not have any significant effect on the percentage of cortical necrosis after transplantation: In kidneys that underwent no NR the median cortical necrotic area was 71% (interquartile range 35–92%) 24 h posttransplant, and for kidneys that were treated with 1 h or 2 h of NR this figure was 53% (interquartile range 36–74%) and 76% (interquartile range 41–95%), respectively (p=0.34). There was no significant interaction between WI time and NR time (p=0.25) for the dependent variable cortical necrosis.

Figure 2a

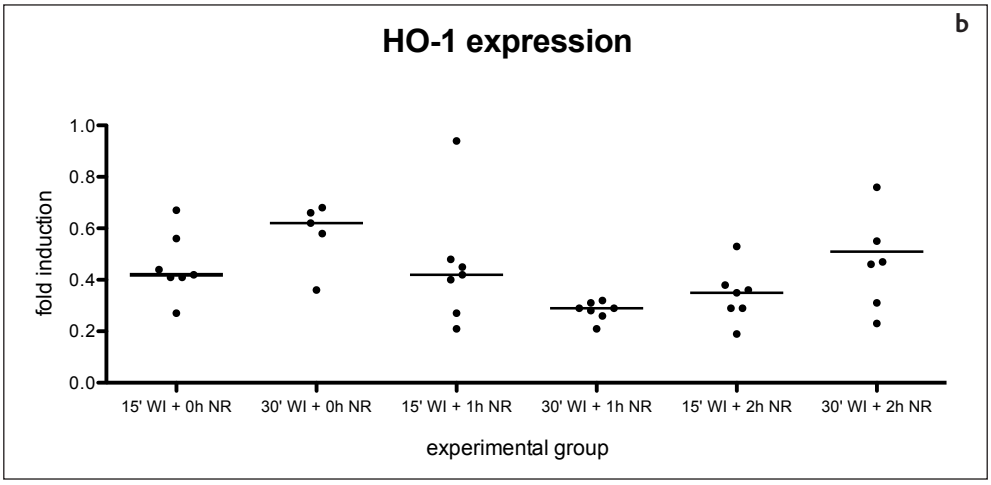


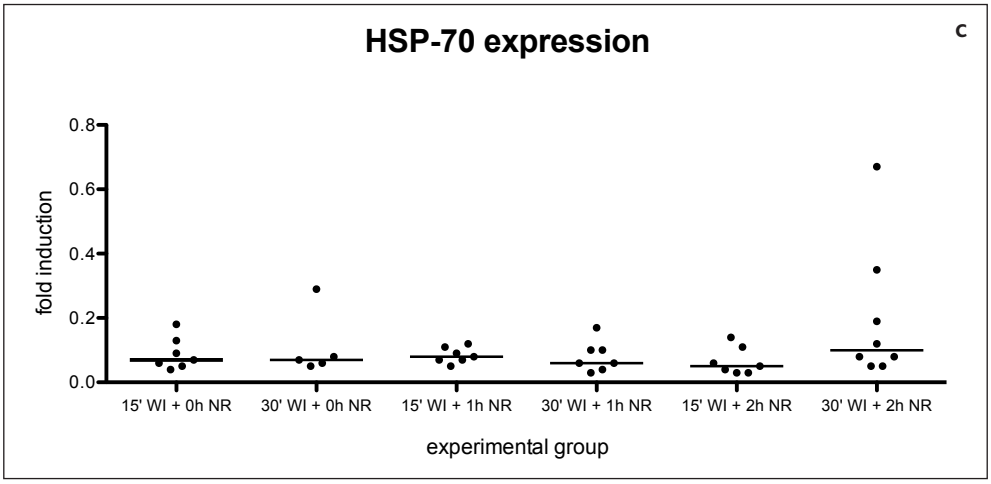
qPCR results

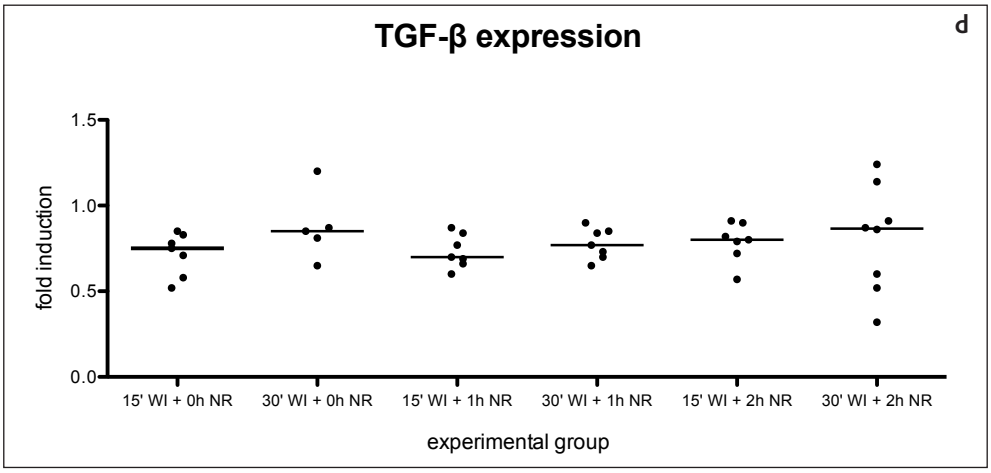
Figures 2b–i present the results of qPCR analyses for each individual transplant, categorized per experimental group. Overall, the expression of genes that are involved in cytoprotection,

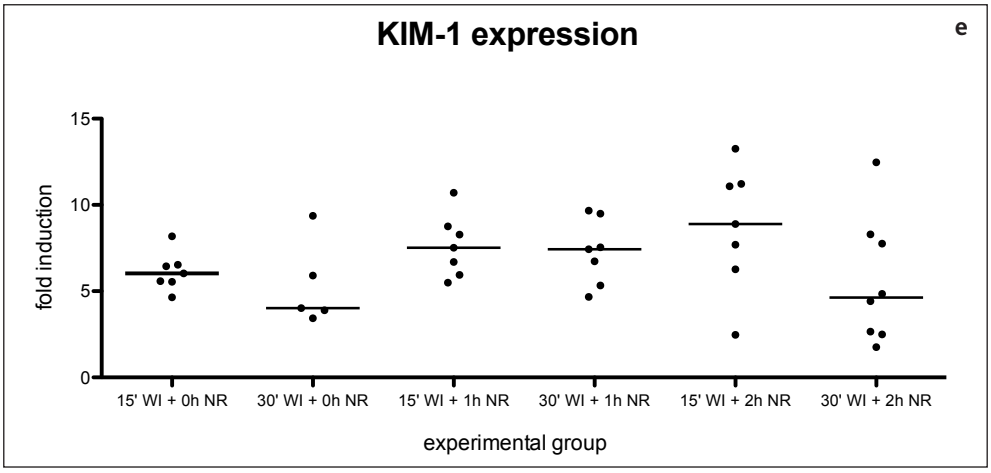
tissue regeneration, tubular injury, inflammation, and interstitial fibrosis was only minimally influenced by WI time and/or NR time. For some markers (KIM-1, HIF-1α, and IL-6), WI time seemed to have a mild influence on gene expression 24 h posttransplant, but none of the tests passed the $p<0.05$ threshold to be qualified as statistically significant ($p=0.10$, $p=0.10$, and $p=0.13$, respectively). Only for HO-1 NR time had a significant influence on gene expression 24 h after transplantation ($p=0.01$). In the post-hoc test, this effect was explained by the difference between no NR and 1 h NR ($p=0.02$), and not by the difference between 1 h and 2 h of NR ($p=0.19$). For all other markers tested, NR did not significantly influence gene expression in the kidney graft 24 h posttransplant.

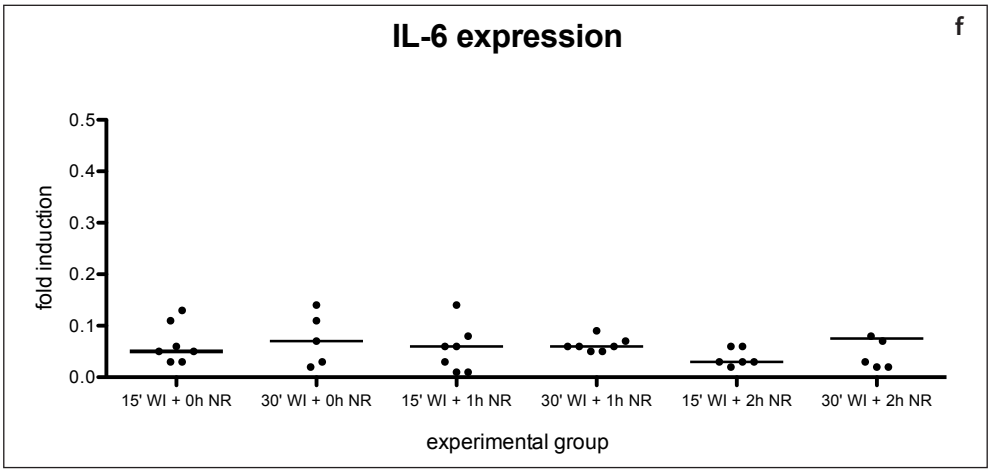
Figure 2b-i






TGF-β expression
d
fold induction
1.5
1.0
0.5
0.0
15' WI + 0h NR
30' WI + 0h NR
15' WI + 1h NR
30' WI + 1h NR
15' WI + 2h NR
30' WI + 2h NR
experimental group


KIM-1 expression
e
fold induction
15
10
5
0
15' WI + 0h NR
30' WI + 0h NR
15' WI + 1h NR
30' WI + 1h NR
15' WI + 2h NR
30' WI + 2h NR
experimental group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fold induction
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0.4
0.3
0.2
0.1
0.0
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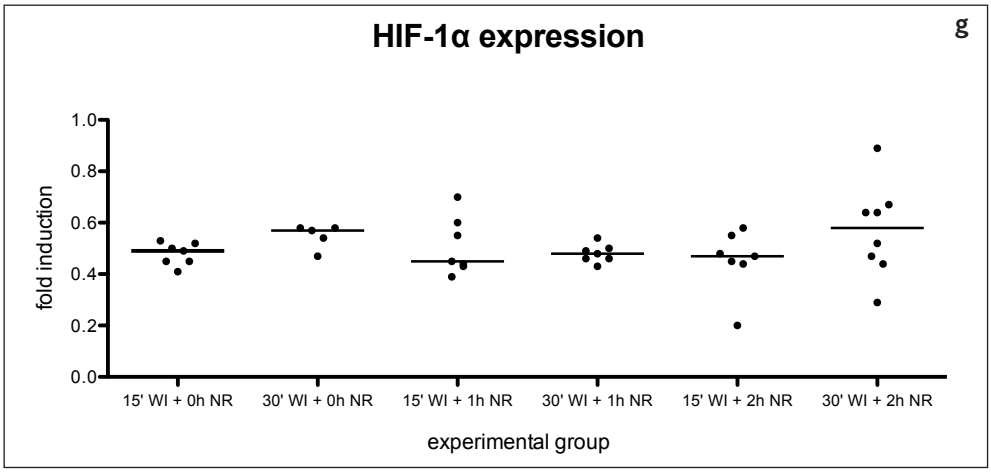
15' WI + 1h NR
30' WI + 1h NR
15' WI + 2h NR
30' WI + 2h NR
experimental group


HIF-1α expression
g
fold induction
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0.8
0.6
0.4
0.2
0.0
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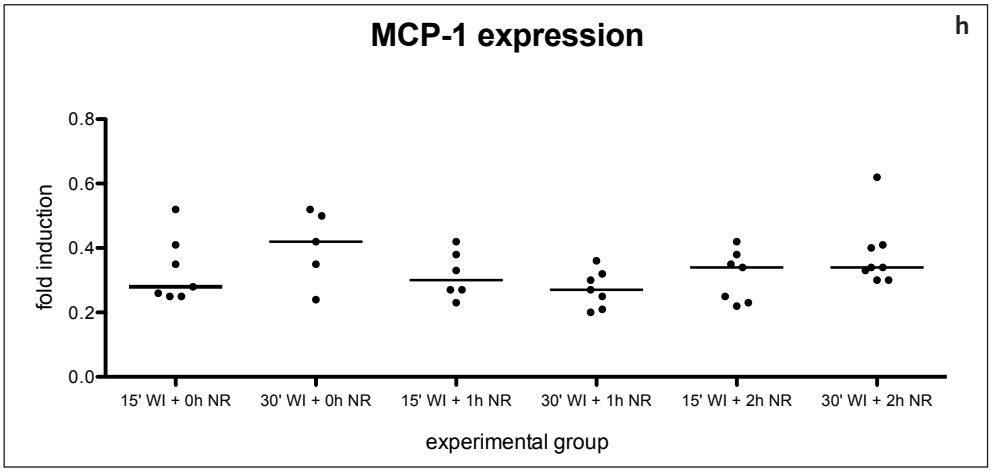
30' WI + 0h NR
15' WI + 1h NR
30' WI + 1h NR
15' WI + 2h NR
30' WI + 2h NR
experimental group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fold induction
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0.6
0.4
0.2
0.0
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30' WI + 0h NR
15' WI + 1h NR
30' WI + 1h NR
15' WI + 2h NR
30' WI + 2h NR
experimental group

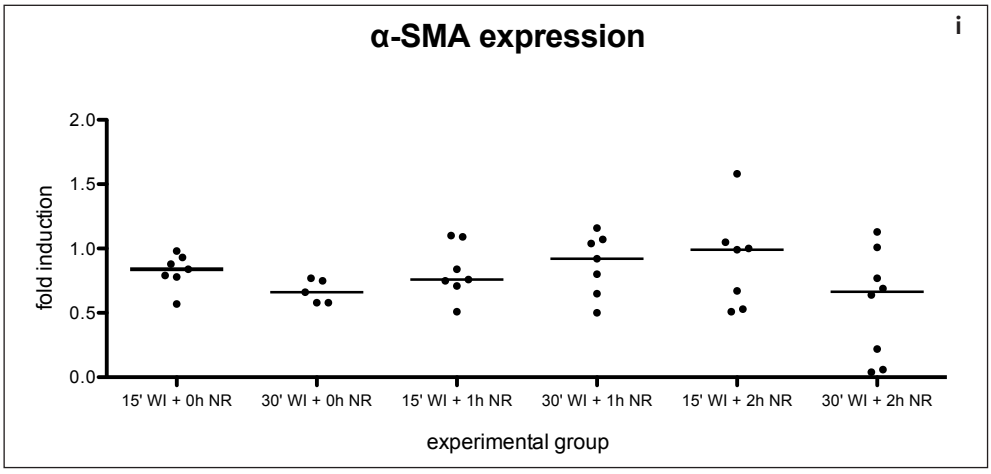
α-SMA expression
i
fold induction
2.0
1.5
1.0
0.5
0.0
15' WI + 0h NR
30' WI + 0h NR
15' WI + 1h NR
30' WI + 1h NR
15' WI + 2h NR
30' WI + 2h NR
experimental group

Figure 2: Percentage of cortical necrosis in kidney grafts 24 h after transplantation **(a)**, and the expression of heme oxigenase-1, heat shock protein-70, transforming growth factor-β, kidney injury molecule-1, interleukin-6, hypoxia inducible factor-1α, monocyte chemoattractant protein-1, and α-smooth muscle actin 24 h after transplantation **(b–i)**. Each black dot represents a single case, and horizontal lines indicate median values per experimental group.

DISCUSSION

Interventions that aim at better preserving donor organ quality prior to transplantation are becoming increasingly important in an era with more marginal deceased donor grafts in the pool[96,97]. Normothermic recirculation immediately after cessation of cardiopulmonary resuscitation measures was initially instituted by the group of the *Hospital Clínic* in Barcelona, Spain to gain extra time to obtain the compulsory judicial permission for uncontrolled (Maastricht category I and II) DCD organ donation.[92] As a side effect, clinicians observed an improved early function of those renal grafts that had been subjected to NR in the donor, compared to kidneys that came from donors whose organs were immediately cooled when cardiopulmonary resuscitation was stopped, with 12.5% versus 75% delayed graft function incidence).[39,94] These findings, together with favorable results of DCD liver transplantation after NR (posttransplant course of uncontrolled DCD livers after NR comparable to that of livers recovered from DBD donors without NR), have led to the hypothesis that NR may somehow resuscitate a DCD donor kidney that has been exposed to severe WI injury.[98] However, to date, the mechanism as well as the magnitude of its postulated effect remains to be unraveled. Interestingly, NR is already clinically utilized at a small scale, although there is no convincing preclinical evidence which supports its principle and/or effectiveness. The aim of the present study was to provide a first piece of such evidence. Much to our surprise, we could not find any indication that NR will somehow protect or resuscitate renal grafts that have sustained profound WI injury. Therefore, the present study does not support the scarce evidence which suggested that NR could have a beneficial effect on kidneys recovered from uncontrolled DCD.

Apart from merely being a preclinical study in a standardized animal model, this study has a few other relevant limitations that should be considered when translating our findings to the human clinical setting. First, in our model WI injury was induced by clamping the renal vessels after systemic heparinization, which is not fully comparable with cardiac arrest followed by cardiopulmonary resuscitation and cessation of such measures as it occurs in human uncontrolled DCD.[40] We chose not to employ a genuine cardiac arrest model, because we wanted to focus on the effect of NR after a clear-cut duration of real WI, avoiding the more complex situation of slowly worsening hypoxia, hypotension, and the associated systemic neurologic and humoral responses that could all have their own isolated effect on the kidney

graft. In addition, we needed a physiologically intact circulation in the donor animal for NR after WI. We employed an auto-NR model, since artificial warm and oxygenated recirculation of small rodents is technically challenging and therefore likely to introduce more variation in our model. A second limitation is that our study lacks functional end points in terms of renal function or graft survival after transplantation. To obtain such data, recipient animals need to stay alive for at least a few weeks posttransplant and the native contralateral kidney of the recipient would have to be removed at the same moment when the donor graft is implanted, or shortly after, to be able to measure early renal function of only the transplanted kidney. Normally, with donor kidneys that have sustained only minimal injury, an orthotopic renal transplant model with native contralateral nephrectomy is easily applicable and rather stable.[99] However, for the present study donor kidneys needed to be severely damaged. Hence, most kidneys would develop delayed graft function in the first days after transplantation. Without a native contralateral kidney in situ, most animals would die of uremia soon after transplantation, or become unacceptably ill in those first days.[95] Dialysis of small rodents is technically very complex and would introduce too much variation in our data. With a native contralateral kidney in situ, as in our model, reliable isolated measurement of graft function is impossible. As a consequence, this study lacks functional end points.

Another limitation of this study could be that we have possibly chosen a too heavy model of WI injury, which rendered most kidneys in a condition that was beyond any recovery. Thus, we may have missed a potential beneficial effect of NR because renal grafts in our study sustained too much damage to be repaired by this intervention. However, even though NR might show some measurable effects in kidneys that are only minimally injured, it is a logistically challenging and rather costly method in humans. We feel that its application is only warranted when NR would lead to a significant improvement of severely damaged donor grafts, which would otherwise not be sufficiently suitable for transplantation.

An earlier preclinical study by Net *et al* showed that, in DCD liver transplantation, the effect of NR could be mediated by a form of ischemic preconditioning.[93] Although proven to be protective against ischemia/reperfusion injury in liver transplantation, ischemic precondition does not seem to have such an effect on kidney grafts.[100] This may in part explain why NR does reduce ischemia/reperfusion related injury in DCD liver grafts, but the method does not significantly protect and/or resuscitates ischemically damaged kidneys.

It is well established that although ischemia itself does lead to tissue injury, subsequent reperfusion will cause even more damage through a multitude of pathways including acute aspecific inflammation and the detrimental effect of reactive oxygen species.[33,101,102] A donor procedure with NR, followed by transplantation of the kidney will follow the sequence warm ischemia – warm oxygenized reperfusion – cold ischemia – warm oxygenized reperfusion and therefore has two instead of just one potentially detrimental episodes of reperfusion. As a consequence, NR might even lead to more instead of less ischemia-reperfusion related kidney injury. In our study, donor kidneys after 30 min of WI and 2 h of NR had significantly more cortical necrosis than renal grafts that had also sustained 30 min of WI, but underwent only 1

h of NR. This finding carefully supports the hypothesis that a long period of NR after profound WI could actually be detrimental to a kidney graft. In addition, in the human setting NR would most likely reperfuse a substantial part of the donor's body, all of which has endured WI. In contrast to our animal model in which only the kidney sustained WI, a human DCD kidney would also be exposed to circulating inflammatory mediators and oxygen free radical that are released upon warm reperfusion of the intestine and the liver.

In conclusion, the present preclinical study could not show any beneficial effect of normothermic recirculation in terms of more cytoprotection, elevated tissue regeneration, less interstitial fibrosis formation, a lower level of aspecific inflammation, or a decreased percentage of tubular necrosis in transplanted kidneys that had sustained severe warm ischemic injury in the donor. Our data do have several relevant limitations which preclude a direct translation to the human clinical setting. Nevertheless, this study by no means supports the concept of normothermic recirculation for DCD kidneys. We feel that more preclinical evidence is needed before this method can be implemented in human uncontrolled DCD, as neither the mechanism nor its effectiveness have been proven and the method might even be detrimental to renal grafts.

ACKNOWLEDGEMENTS

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Chapter 5

Machine perfusion or cold storage in deceased-donor kidney transplantation

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ABSTRACT

Background

Static cold storage is generally used to preserve kidney allografts from deceased donors. Hypothermic machine perfusion may improve outcomes after transplantation, but few sufficiently powered prospective studies have addressed this possibility.

Methods

In this international randomized, controlled trial, we randomly assigned one kidney from 336 consecutive deceased donors to machine perfusion and the other to cold storage. All 672 recipients were followed for 1 year. The primary end point was delayed graft function (requiring dialysis in the first week after transplantation). Secondary end points were the duration of delayed graft function, delayed graft function defined by the rate of the decrease in the serum creatinine level, primary nonfunction, the serum creatinine level and clearance, acute rejection, toxicity of the calcineurin inhibitor, the length of hospital stay, and allograft and patient survival.

Results

Machine perfusion significantly reduced the risk of delayed graft function. Delayed graft function developed in 70 patients in the machine-perfusion group versus 89 in the cold-storage group (adjusted odds ratio, 0.57; P=0.01). Machine perfusion also significantly improved the rate of the decrease in the serum creatinine level and reduced the duration of delayed graft function. Machine perfusion was associated with lower serum creatinine levels during the first 2 weeks after transplantation and a reduced risk of graft failure (hazard ratio, 0.52; P=0.03). One-year allograft survival was superior in the machine-perfusion group (94% vs. 90%, P=0.04). No significant differences were observed for the other secondary end points. No serious adverse events were directly attributable to machine perfusion.

Conclusions

Hypothermic machine perfusion was associated with a reduced risk of delayed graft function and improved graft survival in the first year after transplantation.

INTRODUCTION

Two different forms of organ preservation — static cold storage and hypothermic machine perfusion — are used clinically for renal allografts obtained from deceased donors. In static cold storage, the kidney is flushed, cooled with one of several cold preservation solutions, and transported on ice. In hypothermic machine perfusion, after an initial washout of blood, the kidney is connected to a perfusion device, and a solution is pumped continuously through the renal vasculature at temperatures between 1 and 10°C.[54] The typical deceased kidney donor today is older and has been exposed to more concomitant disease than donors were several decades ago; these factors may have a detrimental effect on allograft quality.[2,18] In addition, the use of organs received from donors after cardiocirculatory death is increasing in most countries.[40] Such allografts are known to have significantly higher rates of delayed graft function.[40,52] Evidence suggests that organs that do not function immediately after transplantation have an increased risk of acute rejection, and allograft survival may be inferior.[31,103] In addition, delayed graft function increases the costs of kidney transplantation.[104,105] Retrospective studies have suggested that machine perfusion could result in a better short-term outcome, with lower rates of delayed graft function after transplantation of kidneys from all types of deceased donors.[46,105,106] Therefore, interest in machine perfusion is increasing. Our international randomized, controlled trial compared machine perfusion with cold-storage preservation in deceased-donor kidney transplantation with a primary end point of delayed graft function.

METHODS

Study design

This investigator-driven, international randomized, controlled study included the Netherlands, Belgium, and the federal state of North Rhine–Westphalia in Germany. All consecutive deceased-donor kidney pairs identified in these regions that met the initial inclusion criteria were eligible for randomization by Eurotransplant, the international organ-exchange organization of Austria, Belgium, Croatia, Germany, Luxemburg, the Netherlands, and Slovenia (Croatia became a member after the present study was completed). Since we aimed to include the whole spectrum of deceased donors, no previous selection of donor types to be included was made. Thus, the study reflects the effect of machine perfusion as compared with cold storage in everyday practice within an international organ-exchange organization. From each donor, one kidney was randomly assigned to machine perfusion and the contralateral organ to cold storage. The organ could be transplanted into any recipient within the Eurotransplant region.[107] Approval for the study was obtained from the ethics review boards in each trial region and from the Eurotransplant Ethical Advisory Committee and Kidney Advisory Committee. Since the random assignment of kidneys to a preservation

method was limited to organs isolated before transplantation, no informed consent from recipients was required for this intervention.

An independent scientific steering committee composed of clinicians and scientists from each trial region was solely responsible for the design, conduct, data analysis, and manuscript preparation for this study.

Inclusion and exclusion criteria

Organ donors had to be 16 years of age or older. Only kidney pairs from deceased donors were included in the study, either from donation after brain death or donation after cardiocirculatory death. The category for donors without a heartbeat had to be Maastricht category III (awaiting cardiocirculatory death after withdrawal of treatment) or IV (cardiocirculatory death in a brain-dead donor).[13] Kidney pairs were included only if both organs were actually transplanted into two different recipients. If one kidney was transplanted into the same recipient together with another organ, this kidney pair was excluded. The only exclusion criterion for recipients was the death of the patient in the first week after transplantation, since a follow-up of at least 1 week was required to determine the primary end point.

Randomization

A randomization scheme based on permuted blocks within regions was used with separate randomization lists for each trial region. A detailed description of the randomization process is available in the Supplementary Appendix, available with the full text of this article at NEJM.org. Surgical teams were allowed to switch preservation methods only if the kidney assigned to machine perfusion had an aortic patch that was too small or if it had too many renal arteries for a reliable connection to the machine-perfusion device; this switch in preservation methods changed the initial randomization.

Logistics

In each trial region, a team of trained perfusionists was on hand 24 hours per day, 7 days per week to respond when a donor became available. The perfusionists transported the machine-perfusion device to the donor hospital and assisted donor surgeons with connecting one kidney to the machine. No changes were made to the existing Eurotransplant rules for organ allocation or to transportation protocols. Kidneys that underwent machine perfusion as well as those that were preserved with cold storage were transported to their respective recipient center without any monitoring.

Hypothermic machine perfusion

LifePort Kidney Transporter machines (Organ Recovery Systems) were used for perfusion, delivering a pulsatile flow of University of Wisconsin machine preservation solution (Kidney Preservation Solution-1)[108] at 1 to 8°C, with no changes in perfusion settings throughout the

preservation period. The systolic perfusion pressure was fixed at 30 mm Hg, and the kidneys underwent machine perfusion from organ procurement until transplantation. To prevent bias in clinical decisions about transplanting or discarding an organ, intravascular resistance and flow readings were never revealed to the transplantation team.

Cold storage

No changes were made to the standard cold-storage protocols. After an initial vascular washout, kidneys were submerged in the preservation solution and stored on melting ice, according to the established Eurotransplant routine.

Data collection

Follow-up data were provided by each participating transplantation center through a secure online database hosted by Eurotransplant. A random sample of 10% of all patients was audited externally; no relevant irregularities were found.

Study end points

The primary end point was delayed graft function, defined as the requirement for dialysis during the first week after transplantation. The secondary end points were the duration of delayed graft function, primary nonfunction (permanent lack of function of the allograft from the time of transplantation), the area under the curve of the daily serum creatinine level at days 1 to 14, the creatinine clearance at day 14, biopsy-proven acute rejection, toxicity of the calcineurin inhibitor, the length of the recipient's hospital stay, and survival of the graft and patient up to 1 year after transplantation. Data on graft survival were censored at the time of death in patients who died with a functioning allograft. In addition to the primary end point, which was defined in terms of the requirement for dialysis after transplantation, we also examined delayed graft function as a secondary end point. This secondary end point, functional delayed graft function, was defined in terms of the absence of a decrease in the serum creatinine level of at least 10% per day for at least 3 consecutive days in the first week after transplantation, not including patients in whom acute rejection, toxicity of the calcineurin inhibitor, or both developed within the first week.[109] All end points described above were prespecified in the study protocol, except primary nonfunction, which was added post hoc.

Statistical analysis

This study was powered to detect a reduction in delayed graft function of at least 10%, based on a presumed incidence of 35% among recipients of kidneys that had been preserved by means of cold storage. With a statistical power of 0.8 and a one-sided type I error of 0.05, the minimum required sample size was 300 kidney pairs; this is equivalent to the required sample size for a logistic-regression analysis with a two-sided type I error of 0.05 and similar power.[110] The primary analysis of the primary end point — delayed graft function — consisted

of a logistic-regression model, which examined whether machine perfusion as compared with cold-storage preservation, in the context of other relevant factors, influenced the risk of delayed graft function.[31,111] Covariates for this model (see the Supplementary Appendix) were prespecified in the study protocol and were based on relevant literature.[112,113] The final model was determined by entering all covariates together in the analysis, with a built-in normal gamma frailty term for the donor to account for the paired study design.[114] For end-point variables, univariate differences between the groups were assessed with the use of McNemar's test or the Wilcoxon signed-rank test. For demographic variables, differences were assessed with the use of Fisher's exact test or the Mann–Whitney test. The Kaplan–Meier method was used to analyze graft and patient survival. Differences between survival curves were determined with the use of log-rank tests. A Cox proportional-hazards model was applied to examine which variables significantly influenced the risk of graft failure.[77] To construct this model, an approach similar to the logistic-regression model for delayed graft function was followed.

We performed prespecified subgroup analyses to determine the treatment effect on the primary end point according to donation after cardiocirculatory death versus donation after brain death and according to expanded-criteria donation versus standard-criteria donation.[115] Expanded-criteria donation was defined as a donor age of 60 years or more or a donor age between 50 and 60 years, with at least two of the following additional donor characteristics: history of hypertension, death due to a cerebrovascular cause, and a serum creatinine level of more than 132 μmol per liter (1.5 mg per deciliter) before removal of the kidney.[12]

All reported P values are two-sided and not adjusted for multiple testing. A P value of 0.05 or less was considered to indicate statistical significance. Analyses were conducted with the use of the SPSS, SAS, and R software packages and were based on all organ pairs that met the inclusion criteria.

No interim analyses of study end points were carried out. At regular intervals, confidential safety analyses were performed by the trial safety board, which compared the reported rates of adverse events between the two trial groups. The sponsor was not involved in the conduct of the study, the analysis or storage of the data, or the preparation of the manuscript. The scientific steering committee vouches for the accuracy and completeness of the data and analyses.

RESULTS

From November 1, 2005, through October 31, 2006, there were 654 potential deceased kidney donors 16 years of age or older in the three trial regions. Figure 1 shows a flow diagram of the 336 kidney pairs (672 recipients) included in our analysis. In 25 donors (4.6%), preservation methods were switched because of the aberrant vascular anatomy of the kidney assigned to machine perfusion. Vascular anomalies were not observed to have a significant effect on delayed graft function. Aberrant vascular anatomy did not significantly increase the risk of graft failure, and the addition of this factor to the Cox model had no effect on the hazard ratio for graft failure associated with machine perfusion versus cold storage (see the Supplementary Appendix).

The 20 "other reasons for exclusion" of the kidney pairs (Figure 1) were as follows: 12 adverse events that occurred during the donor procedure, 5 cases in which the donor had one kidney, 2 cases in which the consent for kidney donation was withdrawn just before procurement, and 1 procedure involving a donor after cardiocirculatory death that was planned as a Maastricht category III donation but was changed to a Maastricht category II donation (cardiocirculatory death after unsuccessful resuscitation).

Study patients

Table 1 summarizes the characteristics of the study groups. All kidneys donated after cardiocirculatory death were in Maastricht category III, as defined earlier. There were no significant differences between the two groups with regard to relevant baseline characteristics.

Table 1

Variable	Machine-perfusion group (N = 336)	Cold-storage group (N = 336)	P value*
Donor characteristics			
Age (yr)			
Median	51		
Range	16–81		
Type of donation (no.)			
After brain death	294		
After cardiocirculatory death	42		
Standard criteria	242		
Expanded criteria	94		
Vascular flush solution (no.)			
University of Wisconsin solution	216		
Histidine–tryptophan–ketoglutarate solution	108		
Euro–Collins solution	1		
Not reported	11		
Recipient characteristics			
Age (yr)			0.21
Median	53	52	
Range	11–79	2–79	
Duration of pretransplantation dialysis (yr)			0.59
Median	4.5	4.4	
Range	0.15–18	0.19–24	
Previous transplants (%)†	23	21	0.38
Panel-reactive antibody level (no.)			0.68
0–5%	297	304	
6–84%	35	29	
>84%	4	3	
Immunosuppressive drugs (%)			
Prednisolone	98	99	0.77
Cyclosporine	50	54	0.25
Tacrolimus	49	46	0.39
Azathioprine	1	2	0.18
Mycophenolate mofetil	86	87	0.73
Antithymocyte globulin	14	13	0.82
Interleukin-2 receptor antagonists	42	47	0.18
Transplant characteristics			
No HLA mismatches (% with no mismatches at the HLA-A, B, or DR loci)	16	15	0.90
Cold ischemic time (hr)			0.30
Median	15.0	15.0	
Range	3.5–29.7	2.5–29.7	
Allograft with >1 renal artery (%)	20	22	0.51

Table 1 Continued

Variable	Machine-perfusion group (N = 336)	Cold-storage group (N = 336)	P value*
Primary end point			
Delayed graft function (%)	20.8	26.5	0.05
Secondary end points			
Functional delayed graft function (%)‡	22.9	30.1	0.03
Primary nonfunction (%)§	2.1	4.8	0.08
Duration of delayed graft function (days)			0.04
Median	10	13	
Range	1–48	1–41	
Creatinine clearance at day 14 (ml/min)			0.25
Median	42	40	
Range	0–171	0–175	
Calcineurin-inhibitor toxicity within 14 days after transplantation (%)	6.3	5.7	0.86
Acute rejection within 14 days after transplantation (%)	13.1	13.7	0.91
Post-transplantation hospital stay (days)			0.78
Median	19	18	
Range	4–392	6–382	

Table 1: Characteristics of donors, recipients, and transplants and univariate differences between the groups.
* For baseline characteristics, P values were calculated with the use of Fisher's exact test for discrete variables and the Mann–Whitney test for continuous variables. For end-point variables, P values were calculated with the use of McNemar's test for discrete variables and the Wilcoxon signed-rank test for continuous variables.
† This category was the percentage of recipients who had undergone one or more renal transplantations before the transplantation included in this analysis.
‡ Functional delayed graft function was defined as the absence of a decrease in the serum creatinine level of at least 10% per day for at least 3 consecutive days in the first week after transplantation. This category did not include patients in whom acute rejection, calcineurin-inhibitor toxicity, or both developed in the first week.
§ Primary nonfunction was defined as the permanent lack of function of the allograft from the time of transplantation.

Delayed graft function

Delayed graft function occurred in 70 recipients in the machine-perfusion group (20.8%) as compared with 89 patients in the cold-storage group (26.5%). Table 2 shows the results of analysis using the logistic-regression model. As compared with cold storage, machine perfusion significantly reduced the risk of delayed graft function (adjusted odds ratio, 0.57; P=0.01).

Subgroup analysis

In September 2006, when enrollment of donors in the study was nearly complete, the scientific steering committee expected that an insufficient number of donors would be enrolled at trial completion to conduct a meaningful subgroup analysis for donation after cardiocirculatory death. At the suggestion of the steering committee and with the permission

of all centers, the inclusion of additional donors after cardiocirculatory death was extended by an amendment to the protocol, until a total of 82 donors were enrolled on August 17, 2007 (see the Supplementary Appendix for details). Solely for the subgroup analysis involving donation after brain death versus donation after cardiocirculatory death, these inclusions were added to the main group of patients to provide more statistical power. Figure 2 shows a forest plot of the treatment effect in the prespecified subgroup analyses. In the main data set, we found no significant difference in the magnitude of the treatment effect on delayed graft function after standard-criteria donation versus expanded-criteria donation (P=0.75) and after donation after brain death versus donation after cardiocirculatory death (P=0.42). In the extended data set, the effect of the preservation method on delayed graft function did not differ significantly between patients who received kidneys from donors after brain death versus patients who received kidneys from donors after cardiocirculatory death (P=0.26).

Secondary end points

Functional delayed graft function occurred in 77 recipients in the machine-perfusion group and in 101 recipients in the cold-storage group (22.9% vs. 30.1%, P=0.03). The incidence of primary nonfunction in the cold-storage group (4.8% vs. 2.1%, P=0.08) was more than two times higher than in the machine-perfusion group, but this difference did not reach statistical significance. If delayed graft function developed, its duration was 3 days shorter after machine perfusion as compared with cold storage (10 days vs. 13 days, P=0.04). There were no significant differences between the study groups in creatinine clearance at 14 days after transplantation, length of hospital stay of recipients, the incidence of toxicity of the calcineurin inhibitor, and acute rejection rate in the first 14 days after transplantation. Daily serum creatinine values in the first 2 weeks after transplantation were significantly lower in recipients in the machine-perfusion group than in recipients in the cold-storage group (median area under the curve, 1456 [range, 385 to 5782] vs. 1787 [range, 288 to 6500]; P=0.01) (see Fig. S2 in the Supplementary Appendix).

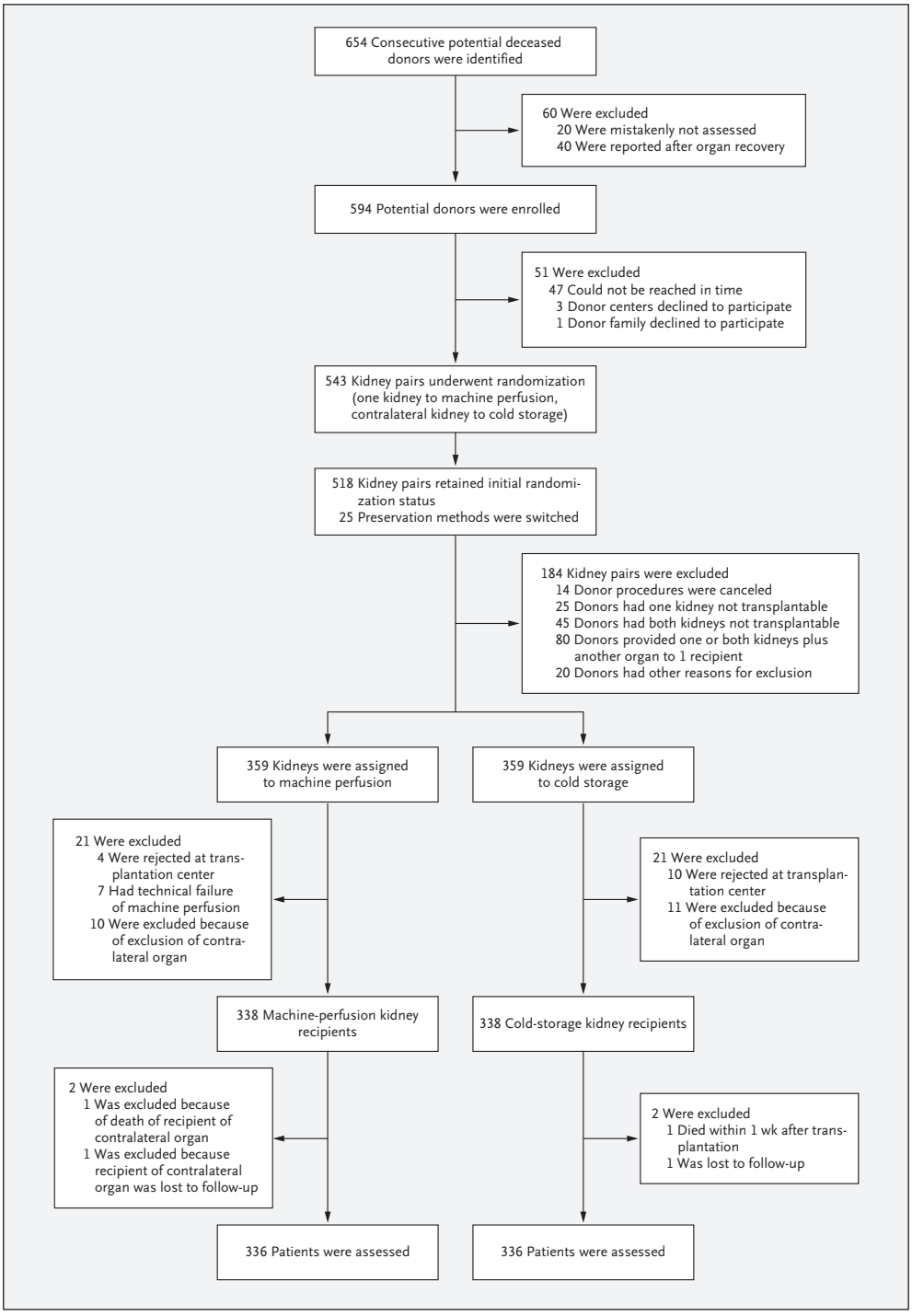


Figure 1: Enrollment, assignment of kidney pairs to machine perfusion or cold storage, follow-up, and assessment.

Variable	Odds ratio (95% CI)	Hazard ratio (95% CI)	P-value
Delayed graft function			
Machine perfusion vs. cold storage	0.57 (0.36–0.88)		0.01
Panel-reactive antibody level — %	1.01 (0.99–1.02)		0.29
Recipient age — yr	1.01 (0.99–1.03)		0.28
Donor age — yr	1.03 (1.00–1.06)		0.04
ECD donor vs. SCD donor†	1.04 (0.46–2.34)		0.92
Cold ischemic time — hr	1.08 (1.03–1.14)		0.003
HLA mismatches — no.	1.13 (0.94–1.37)		0.18
Duration of pretransplantation dialysis — yr	1.16 (1.03–1.31)		0.01
Second or later transplantation vs. first transplantation	3.01 (1.75–5.18)		<0.001
DCD donor vs. DBD donor	17.2 (8.16–36.2)		<0.001
Graft failure within 1 yr after transplantation‡			
Machine perfusion vs. cold storage		0.52 (0.29–0.93)	0.03
DCD donor vs. DBD donor		0.90 (0.28–2.92)	0.87
Recipient age — yr		0.97 (0.95–1.00)	0.02
Duration of pretransplantation dialysis — yr		1.00 (0.87–1.15)	0.97
Panel-reactive antibody level — %		1.01 (0.99–1.03)	0.31
Cold ischemic time — hr		1.04 (0.97–1.11)	0.25
Donor age — yr		1.05 (1.01–1.10)	0.02
ECD donor vs. SCD donor†		1.18 (0.42–3.27)	0.76
HLA mismatches — no.		1.23 (0.98–1.55)	0.08
Second or later transplantation vs. first transplantation		1.72 (0.88–3.35)	0.11

Table 2: Multivariate analysis of the risk of delayed graft function and graft failure.*

* A logistic-regression model was used to determine the odds ratio for delayed graft function, and a Cox proportional-hazards model was used to determine the hazard ratio for graft failure. Odds ratios and hazard ratios are associated with a 1-unit increase in each covariate. CI denotes confidence interval, DBD donation after brain death, DCD donation after cardiocirculatory death, ECD expanded-criteria donation, and SCD standard-criteria donation.

† Since donor age was a separate covariate in these models and donor age was also part of the ECD definition, the effect of ECD versus SCD on delayed graft function and the risk of graft failure may appear to be less pronounced than commonly reported.

‡ Data on graft survival were censored at the time of death in patients who died with a functioning allograft.

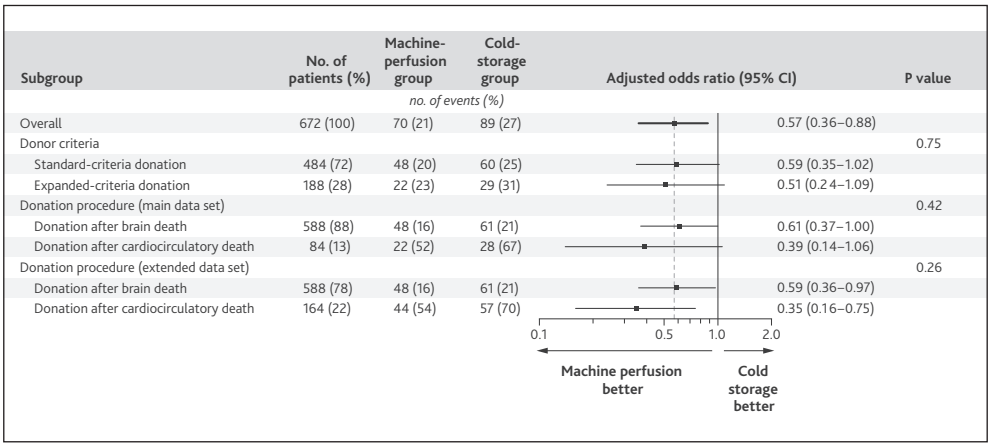


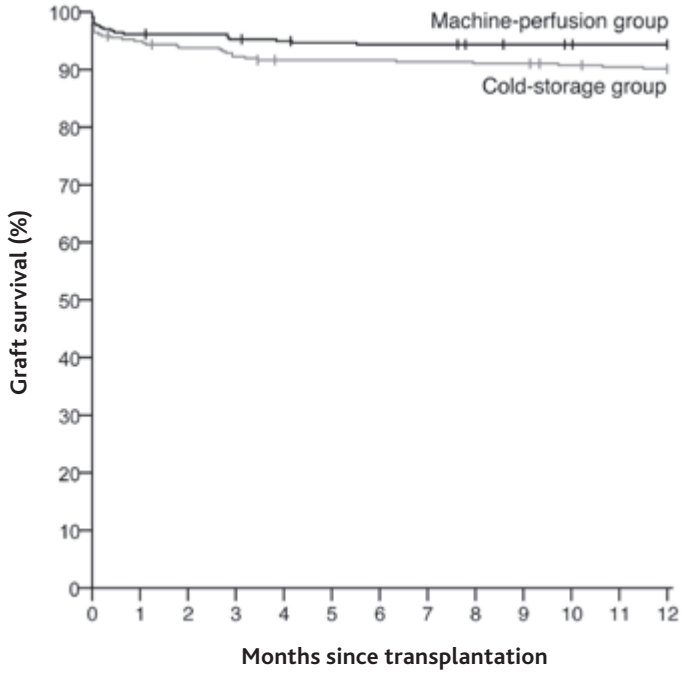
Figure 2: Forest plot of the treatment effect in prespecified subgroup analyses.
In the main data set, there was no significant difference in the magnitude of the treatment effect on delayed graft function after standard-criteria donation versus expanded-criteria donation (P=0.75) and after donation after brain death versus donation after cardiocirculatory death (P=0.42). The extended data set consisted of the main data set plus the additional 80 recipients of kidneys from donors after cardiocirculatory death who were enrolled after the inclusions had ended. This extended data set of 752 recipients was used solely to provide more statistical power for a meaningful subgroup analysis of donation after cardiocirculatory death versus donation after brain death. In the extended data set, the effect of the preservation method on delayed graft function did not differ significantly between patients who received kidneys from donors after brain death versus patients who received kidneys from donors after cardiocirculatory death (P=0.26). P values are for the interaction between the treatment effect (machine perfusion vs. cold storage) and any subgroup variable.

Patient and graft survival

In the cold-storage group, one patient died within 1 week after transplantation because of cardiac arrhythmia and was therefore excluded from the study along with the recipient of the contralateral kidney. At 1 year after transplantation, patient survival was 97% in both groups. Between 7 days and 1 year after transplantation, 11 patients in the machine-perfusion group died and 9 patients in the cold-storage group died (Table 3). One-year graft survival (Figure 3) in the machine-perfusion group was significantly higher than in the cold-storage group (94% vs. 90%, P=0.04). Cox regression analysis (Table 2) showed that machine perfusion significantly reduced the risk of graft failure in the first year after transplantation, with a hazard ratio of 0.52 (P=0.03). A post hoc analysis in which delayed graft function was added as a time-dependent covariate to the Cox model indicated that recipients with delayed graft function had a significantly increased risk of graft failure (hazard ratio, 1.69; P<0.001); when this was applied, the hazard ratio for graft failure with machine perfusion versus cold storage increased to 0.60, and this covariate became nonsignificant in the model (P=0.08) (see the Supplementary Appendix).

Adverse events

Table 3 summarizes reported adverse events and deaths. No serious adverse events directly attributable to machine perfusion were observed.



No. at risk													
Machine-perfusion group	336	323	322	319	317	315	314	314	312	311	310	309	309
Cold-storage group	336	318	313	308	304	304	304	303	302	302	299	299	296

Figure 3: Graft survival after transplantation.
The rate of graft survival at 1 year in the machine-perfusion group was significantly higher than the rate in the cold-storage group (94% vs. 90%, P=0.04). Data on graft survival were censored at the time of death in patients who died with a functioning allograft.

Table 3

Variable	Machine-perfusion group (N = 336)	Cold-storage group (N = 336)
Adverse events	*no. of events (%)*	
During donor procedure[†]		
Vascular anatomy of both kidneys unsuitable for machine perfusion	7 (2)	
Surgical team insisted on using machine perfusion for both kidneys	4 (1)	
Surgical team declined to cooperate with study	3 (1)	
13-yr-old donor mistakenly underwent randomization	1 (<1)	
Renal polar artery overlooked during procurement[‡]	1 (<1)	
During organ preservation		
Technical failure or malfunction during machine perfusion[§]	7 (2)	NA
Delayed delivery of cross-match material[¶]	1 (<1)	0
Serious — in recipients		
Any serious event	77 (23)	88 (26)
Severe urinary tract infection	11 (3)	10 (3)
Sepsis due to any cause	9 (3)	10 (3)
Diabetes mellitus	9 (3)	10 (3)
Severe respiratory tract infection	8 (2)	14 (4)
Postoperative bleeding	8 (2)	8 (2)
Peritonitis	6 (2)	5 (1)
Any arterial thrombosis	6 (2)	4 (1)
Any venous thrombosis	6 (2)	4 (1)
Any cancer	4 (1)	9 (3)
Severe gastrointestinal tract infection	4 (1)	5 (1)
Cardiac decompensation	3 (1)	3 (1)
Myocardial infarction	2 (1)	2 (1)
Ileus	1 (<1)	3 (1)
Gastrointestinal bleeding	0	2 (1)
Minor — in recipients		
Any minor event	170 (51)	148 (44)
Uncomplicated urinary tract infection	43 (13)	47 (14)
Cytomegalovirus infection or reactivation of infection	23 (7)	29 (9)
Uncomplicated gastrointestinal tract infection	22 (7)	21 (6)
Seroma	20 (6)	13 (4)
Ureteral stenosis (graft)	12 (4)	5 (1)
Anemia	11 (3)	8 (2)
Electrolyte disturbances	9 (3)	5 (1)
Leukopenia	7 (2)	2 (1)
Wound abscess	5 (1)	3 (1)
Hydronephrosis of unknown cause (graft)	5 (1)	2 (1)
Mild cardiac arrhythmia	5 (1)	2 (1)
Incisional hernia	4 (1)	5 (1)
Upper respiratory tract infection	2 (1)	6 (2)
Renal capsular hematoma due to biopsy[‖]	2 (1)	NA

Table 3 continued

Variable	Machine-perfusion group (N = 336)	Cold-storage group (N = 336)
Deaths	*no. of events*	
Any cause	11	9
Multiorgan failure due to sepsis	4	2
Gastrointestinal bleeding	2	0
Death from unknown cause	2	0
Pneumonia	1	2
Malignant condition	1	1
Pulmonary embolism	1	0
Myocardial infarction	0	2
Cerebral abscess	0	1
Uncontrolled bleeding	0	1

Table 3: Adverse events and deaths reported in the first year after transplantation.*

* All serious adverse events except the study end points are listed in this table. No serious adverse events directly attributable to machine perfusion were reported. Of all minor adverse events, only those that occurred in 1% or more of all patients are listed. No statistical tests were performed on the data in this table. NA denotes not applicable.

† All these events led to exclusion of the kidney pair from the study.

‡ One kidney was unsuitable for transplantation because of the insufficient length of the remaining polar artery.

§ None of these events rendered the graft unsuitable for transplantation. When machine perfusion failed, the kidney was automatically preserved by means of cold storage inside the machine.

¶ Transplantation was postponed for 3 hours because of a delayed cross-match.

‖ For an amendment to the study protocol that addressed additional research questions not reported in this article, cortical-biopsy specimens were obtained from several machine-perfused kidneys. Capsular hematomas did not compromise the function of these kidneys.

DISCUSSION

Static cold storage is the easiest and most widely used preservation method in kidney transplantation. In the United States, it is used in 80% of these procedures, and in Eurotransplant countries it is used in approximately 100%.[36,91] Although retrospective studies have suggested that machine perfusion is superior,[46,105,106] these registry analyses are biased because of the selection of donor kidneys to be perfused or allografts that are discarded on the basis of perfusion variables. Several prospective studies have either lacked adequate randomization or have had equivocal results because of small sample sizes.[116-120] The present study indicates that machine perfusion significantly reduces the risk of delayed graft function; these findings are probably related to the study's size and strictly paired design.

The relatively large number of exclusions in our study is typical for a paired study in organ

preservation, since logistics necessitated that randomization occur at a very early stage in the donation cascade, when a patient in an intensive care unit (ICU) was a potential kidney donor. Only after both kidneys had actually been transplanted could we determine whether a donor would meet the inclusion criteria. The exclusion of donors from whom one kidney was discarded may have led to a mild bias toward the "better" kidney donors in our study. The same might be true regarding donors who were not included because the donor hospital could not be reached in time by the perfusionist. Theoretically, such donors may have been patients in the ICU who had more unstable conditions. Conversely, excluding donors from whom combined kidney–pancreas transplantations were performed may have slightly biased the data in the opposite direction, since, in general, only the most optimal donors are considered for these procedures. In a small number of patients, the initial randomization was switched because of the vascular anatomy. It is unlikely that this practice has significantly biased the study's outcomes, since aberrant vascular anatomy did not have a significant effect on delayed graft function or on the risk of graft failure, and the observed effect of the machine perfusion versus cold-storage covariate did not change when this factor was added to the Cox model.

The effect of machine perfusion on delayed graft function in our study is slightly stronger than the associations observed in retrospective studies and meta-analyses (odds ratios, 0.62 to 0.73).[46,105] The median cold ischemic time in both treatment groups was relatively short as compared with that in other data sets[36]; this may explain why the incidence of delayed graft function in the cold-storage group in this study was 8.5% lower than the originally anticipated incidence of 35.0%. In addition, the effect of machine perfusion may have been stronger if cold ischemic times had been longer.[36] Machine perfusion was associated with a more pronounced decrease in functional delayed graft function than that observed in the primary end point. Hence, the magnitude of the beneficial short-term effect of machine perfusion may, in part, depend on how delayed graft function is defined.

The treatment effect on the primary end point did not differ between subgroups of deceased donors. On the basis of the evidence from this and other studies,[106] it is probably most legitimate to assume that the effect of machine perfusion as compared with cold storage on delayed graft function is at or near the overall odds ratio of 0.57 in various subgroups. With this assumption, machine perfusion can be considered to have a beneficial effect on the short-term outcome in all common types of deceased-donor kidney transplantation. Nevertheless, there is a higher incidence of delayed graft function among recipients of kidneys donated after cardiocirculatory death and with expanded-criteria donation.[121] Hence, the absolute number of patients who would actually benefit from machine perfusion might be larger in these subgroups.

Machine perfusion was associated with a significant decrease in graft loss, which became apparent within 1 year after transplantation. The post hoc addition of delayed graft function as a covariate to the Cox model suggests that delayed graft function renders a kidney recipient

more at risk for graft failure. In addition, it was linked to an increase in the hazard ratio for graft failure associated with machine perfusion versus cold storage, and this covariate became nonsignificant in the model. Therefore, we think that the reduction in delayed graft function associated with machine perfusion contributes to the improvement in graft survival.

The number of patients with primary nonfunction was reduced by half in the machine-perfusion group as compared with the cold-storage group. However, this difference was not statistically significant, which may be explained by the low overall incidence of primary nonfunction. In this trial, characteristics of machine perfusion were not allowed to be used as a diagnostic tool to identify kidneys that were at risk for a poor outcome. Although evidence is scarce, attention to these variables, as well as to perfusate viability markers, might further increase the effect of machine perfusion on transplantation outcomes.[122]

In conclusion, the present trial showed that hypothermic machine perfusion reduced the incidence of delayed graft function in the kidneys obtained from the most common types of deceased donors. In addition, machine perfusion reduced the duration of delayed graft function, when it occurred. Machine-perfused renal allografts had a lower risk of graft failure in the first year after transplantation and, as a result, these kidneys showed an improved 1-year graft survival as compared with kidneys preserved by static cold storage.

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SUPPLEMENTARY APPENDIX

List of participating transplant centers (centers and names)

Austria (4 centers, 17 recipients) – **Landeskrankenhaus Graz:** H. Müller, H. Holzer; **Universitätsklinik für Chirurgie Innsbruck:** R. Margreiter, C. Bösmüller, W. Mark, H. Fetz; **Allgemeines Krankenhaus der Stadt Linz:** C. Gross, B. Schmekal; **Allgemeines Krankenhaus Wien:** F. Mühlbacher, I. Kristo, M. Pones, G. Györi.

Belgium (7 centers, 183 recipients) – **Universitair Ziekenhuis Antwerpen:** D. Ysebaert, J.L. Bosmans, G. Van Beeumen, W. Van Donink; **Universitair Ziekenhuis Brussel:** J. Lamote, J. Sennesael, B. Amerijckx; **Hôpital Erasme Bruxelles:** A.D. Hoang, D. Mikhalski, D. Abramovicz, V. Brulein; **Universitair Ziekenhuis Gent:** C. Randon, P. Peeters, M. VanderVennet; **Cliniques Universitaires St. Luc Bruxelles:** M. Mourad, M. de Meyer, J. Malaise, L. De Pauw; **Centre Hospitalier Universitaire Liège:** J.P. Squifflet, L. Weekers, O. Detry, M.H. Delbouille; **Universitaire Ziekenhuizen Leuven:** J. Pirenne, Y. Vanrenterghem, F. van Gelder, B. Desschans.

Germany (38 centers, 327 recipients) – **Universitätsklinikum Aachen:** G. Jakse, D. Rohrmann, J. Floege, A. Homburg; **Knappschaftskrankenhaus Bochum:** R. Viebahn, O. Vonend, P. Schenker, A. Wunsch; **Universitätsklinik Bonn:** S.C. Müller, H. Klehr; **Universitätsklinikum Düsseldorf:** W. Sandmann, K. Ivens, A. Voiculescu, K. Balser; **Universitätsklinikum Essen:** A. Paul, O. Witzke, J. Treckmann, A. Jonait-Borkenhagen; **Medizinische Universitätsklinik Köln-Lindenthal:** D. Stippel, Th. Benzing, K. Prenzel, B. Hoppe; **Städtische Krankenanstalten Köln-Merheim:** M. Ströhlein, W. Arns, R. Hackenberg, U. Lange; **Westfälische WU Klinikum Münster:** H. Wolters, B. Suwelack; **Zentralklinikum Augsburg:** E. Nagel, H. Weihprecht, R. Eser, T. Breidenbach; **Charité Berlin - Campus Benjamin Franklin:** K. Miller, M. Van der Giet, E. Krusic, M. Tölle; **Charité Berlin - Campus Mitte:** F. Fuller, K. Budde; **Charité Berlin - Campus Virchow:** J. Pratschke, P. Reinke, Th. Mehlitz; **Zentralkrankenhaus Bremen:** S. Melchior, F.A. Zantvoort, Ch. Bahrs, S. Meier; **Universitätsklinikum Carl Gustav Carus Dresden:** M. Wirth, P. Gross, S. Leike, J. Passemer; **Klinikum der JW Goethe Universität Frankfurt:** M. Probst, E.-H. Scheuermann; **Klinikum der AL Universität Freiburg:** P. Pisarski, P. Gerke, M. Geyer, S. Hils; **Universitätsklinikum Halle:** A. Hamza, O. Rettkowski, K. Fischer, A. Haberland; **Klinikum der Universität Heidelberg:** J. Schmidt, M. Zeier, B. Schmied, C. Sommerer; **Nephrologisches Zentrum Niedersachsen:** J. Küster, V. Kliem; **Medizinische Hochschule Hannover:** F. Lehner, A. Schwarz, M. Hiss, N. Mogilewskaja; **Universitätsklinik des Saarlandes Homburg/Saar:** M. Stöckle, M. Girndt, M. Janssen, U. Sester; **Medizinische Fakultät/Klinikum Jena:** Th. Steiner, O.H. Undine, J. Schubert, G. Wolf; **Universitätsklinikum Schleswig-Holstein Kiel:** D.C. Bröring, U. Kunzendorf, P. Glass, F. Braun; **Westpfalz-Klinikum**

Kaiserslautern: W. Seybold-Epting, Th. Rath, A. Dahms; **Universitätskrankenhaus Leipzig:** J. Hauss, P. Martin, D. Weinert; **Universitätsklinikum Schleswig-Holstein Lübeck:** C. Bürk, M. Nitschke; **Klinikum der Stadt Mannheim:** M. Schwarzbach, P. Schnülle; **Klinikum Rechts der Isar München:** M.C. Raggi; **Klinikum Grosshadern München:** W.-D. Illner, M. Rentsch; **Klinikum Lahnberge Marburg/Lahn:** J. Geks, U. Kuhlmann, T. Maier, J. Hoyer; **Klinikum der Joh. Gutenberg Universität Mainz:** J. Thüroff, O. Schreiner, J. Jones, K. Allers; **Universitätskrankenhaus Erlangen-Nürnberg:** G. Schott, Ch. Hugo, K. Pressmar, K. Hirsch; **Medizinische Fakultät Rostock:** K. Stein, M. Burde; **Katharinenhospital Stuttgart:** M. Schock, G. Hasche, Ch. Olbricht, M. Kalus; **Chirurgische Universitätsklinik Tübingen:** W. Steurer, N. Heyne, Ch. Thiel, K. Knubben; **Universitätskrankenhaus Ulm:** J. Mayer, F. Keller, C. Brockschmidt, S. Stracke; **Klinikum der Bayerischen J-M-U Würzburg:** K. Lopau, R. Bonfig.

Luxemburg (1 center, 4 recipients) – **Centre Hospitalier de Luxembourg:** S. Lamy, P. Duhoux, E. Tasch, J. De Sousa.

The Netherlands (8 centers, 136 recipients) – **Academisch Medisch Centrum Amsterdam:** M.M. Idu, F.J. Bemelman, I. ten Berge, K. van Donselaar; **Universitair Medisch Centrum Groningen:** H.S. Hofker, V.B. Nieuwenhuijs, C. Krikke, M. van Dijk; **Leids Universitair Medisch Centrum:** J. Ringers, A.F.M. Schaapherder, J.W. de Fijter, J. Dubbeld; **Academisch Ziekenhuis Maastricht:** E. van Heurn, J. van Hooff, M. Christiaans; **UMC St. Radboud Nijmegen:** J.A. van der Vliet, A.J. Hoitsma; **Erasmus Medisch Centrum Rotterdam:** J.N.M. Yzermans, W. Weimar, J. Kal-van Gestel; **Universitair Medisch Centrum Utrecht:** R.W.H. van Reedt Dortland, R.J. Hené, V. Leydekkers, C. van Straalen; **Wilhelmina Kinderziekenhuis Utrecht:** M.R. Lilien.

Slovenia (1 center, 5 recipients) – **University Medical Center Ljubljana:** D. Kovac.

Ethics committee approval

Approval for the study was obtained from ethical review boards in each trial region, and from the Eurotransplant Ethical Advisory Committee and Kidney Advisory Committee. As the randomized intervention was limited to isolated organs before transplantation, according to national laws no informed consent from recipients was required. In addition, ethical rationale for not requiring informed consent for the organ preservation method was as follows: At the moment of randomization, as well as at the time point at which the randomized intervention had to be initiated, most kidneys woud not yet be allocated to a potential recipient. Therefore, it would not be possible to obtain informed consent from the recipient before these important time points. As soon as organ allocation was known, no informed consent could be obtained from the potential recipient, as most national laws dictate a 24-hour consideration period, and it would be medically unacceptable to delay transplantation for this reason only. Moreover, should the potential recipient decide not to give informed consent, this would automatically imply that he or she would not receive the kidney, as the randomized intervention was already in progress at that moment. This would lead to an ethically unacceptable dilemma.

Two ethics committees (University Hospital Ghent and University Hospital of Leuven, Belgium) ruled that informed consent would be required to obtain follow-up data from recipients after transplantation in these hospitals, as this was a prospective study. This decision was respected by the steering committee. Other ethics committees did not require specific informed consent for this study's follow-up data retrieval, since the prospective randomized intervention was limited to isolated organs before allocation, and no more than standard clinical data were collected retrospectively without additional requirements due to the study that would affect the patient.

Randomization process

To avoid regional imbalances between the two study arms due to slightly different allocation algorithms, a randomization scheme based on permuted blocks within regions was used with separate randomization lists for each of the three trial regions. Randomization lists were available only to the 24-hour Eurotransplant duty desk. Upon report of a kidney donor, the allocation officer first checked its eligibility and then assigned the left kidney to treatment with either machine perfusion or cold storage following one of the three randomization schemes, which automatically assigned the right kidney to preservation with the other method. Then both kidneys were offered according to the match list, without revealing the preservation method. Only if the kidney assigned to be machine perfused had a too small aortic patch or too many renal arteries preventing a reliable connection to the machine perfusion device were surgical teams allowed to switch preservation methods during organ procurement, thus frustrating randomization.

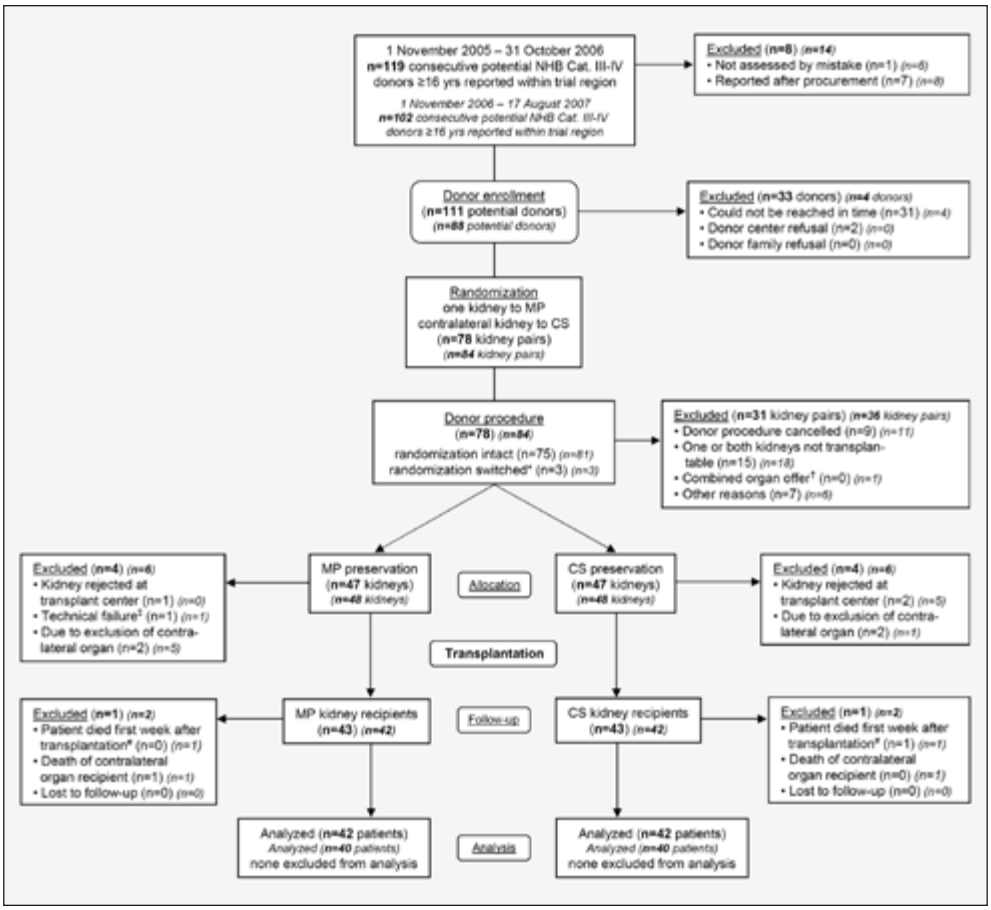


Figure S1: Enrollment, group assignment, follow-up and analysis of non-heart beating donor inclusions. Text and numbers in italics refer to the second era of non-heart beating donor inclusions, after main consecutive inclusions (heart beating and non-heart beating mixed) had ended. These extra non-heart beating donor cases were solely used for a subgroup analysis of the effect size of machine perfusion versus cold storage in non-heart beating versus heart beating kidney grafts. NHB denotes non-heart beating, HB heart beating, MP machine perfusion, and CS cold storage.

* Switching randomization was only allowed when vascular anatomy made one kidney less suitable for the machine.

† Whenever one or both kidneys were offered together with another organ to one recipient, e.g. for combined pancreas–kidney or liver–kidney transplantation.

‡ None of these failures rendered the graft unsuitable for transplantation. When machine perfusion failed, the kidney was automatically cold stored inside the machine.

Causes of death were one transplant related and two non-transplant related events.

Table S1

Variable	Era 1 (1 November 2005 – 31 October 2006)			Era 2 (1 November 2006 – 17 August 2007)			
	Machine perfusion arm (N = 42)	Cold storage arm (N = 42)	P value (Machine perfusion vs. cold storage)	Machine perfusion arm (N = 40)	Cold storage arm (N = 40)	P value (Machine perfusion vs. cold storage)	P value (Era 1 vs. Era 2)
Donor demographics							
Donor age (yr)	41 (17–60)		-	48 (20–67)		-	<0.001
Maastricht category (III / IV)	42 / 0		-	40 / 0		-	-
SCD / ECD	39 / 3		-	29 / 11		-	0.02
Recipient demographics							
Recipient age (yr)	49 (24–69)	51 (27–77)	0.66	48 (27–73)	53 (24–76)	0.94	0.45
Duration of pre-transplant dialysis (yr)	4.7 (1.1–18)	4.4 (1.1–11)	0.30	3.7 (1.0–10)	3.5 (0.36–8.8)	0.74	0.002
Previous transplants (%)†	19	19	0.61	0	0	-	-
Panel-reactive antibodies (0–5% / 6–84% / >84%)	90 / 10 / 0	81 / 17 / 2	0.17	83 / 17 / 0	93 / 7 / 0	0.16	0.46
Prednisolon (%)	98	98	-	95	100	0.25	0.67
Cyclosporin (%)	50	50	-	38	25	0.17	0.02
Tacrolimus (%)	50	52	0.50	53	75	0.03	0.12
Azathioprine (%)	2	2	-	0	0	-	-
Mycophenolate mofetil (%)	88	86	0.53	93	83	0.25	0.55

Anti-thymocyte globulin (%)	10	10	-	20	20	-	0.08
Interleukin-2 receptor antagonists (%)	31	40	0.25	45	25	0.05	0.53
Transplant demographics							
Human leukocyte antigen mismatches (% of 0 mismatches)‡	2	0	0.10	3	8	0.83	0.31
Cold ischemic time (h)	16 (4–25)	16(10–23)	0.54	15(10–29)	18 (9–47)	0.41	0.70

Table S1: Demographics of both eras of non-heart beating donor inclusions.*

* If not indicated otherwise, values are expressed as median (range). P values were obtained by Fisher's exact test for discrete variables and by the Mann–Whitney test for continuous variables. SCD denotes standard criteria donation, and ECD expanded criteria donation.

† Indicates the percentage of recipients who had undergone one or more renal transplants prior to the one included in this analysis.

‡ Indicates the percentage of transplants with zero human leukocyte antigen A/B/DR mismatches.

Trial safety board

To prevent any bias in clinical decisions about transplanting or discarding an organ, machine perfusion dynamics data – such as intravascular resistance and flow readings – were never revealed to the transplant team. A safety board of experienced transplant surgeons was established and consulted on three occasions: In two out of three cases it felt the need to reveal machine perfusion dynamics data to the recipient center. In both of these cases the recipient centers saw no reason to discard the organ based on the additional information and transplanted the kidney.

Prespecified covariates for the logistic regression and cox regression analysis

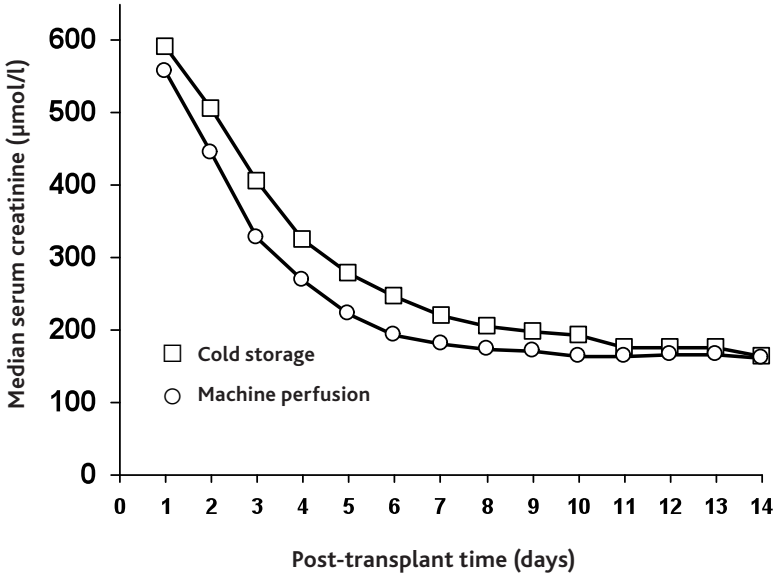
- Machine perfusion vs. cold storage
- Panel-reactive antibody level (%)
- Recipient age (yr)
- Donor age (yr)
- Expanded criteria donor vs. standard criteria donor
- Cold ischemic time (h)
- HLA mismatches (no.)
- Duration of pretransplantation dialysis (yr)
- Retransplantation vs. first transplantation
- Non-heart beating donor vs. heart beating donor

Variable	Hazard ratio (95% confidence interval)	P value
Delayed graft function		
Machine perfusion vs. cold storage	0.59 (0.38–0.92)	0.02
Aberrant vascular anatomy†	1.00 (0.56–1.77)	0.98
Panel-reactive antibody level (%)	1.01 (0.99–1.02)	0.28
Recipient age (yr)	1.01 (0.99–1.03)	0.37
Donor age (yr)	1.03 (1.00–1.06)	0.05
ECD donor vs. SCD donor	1.08 (0.47–2.45)	0.86
Cold ischemic time (h)	1.08 (1.02–1.14)	0.005
HLA mismatches (no.)	1.12 (0.93–1.35)	0.23
Duration of pre-transplant dialysis (yr)	1.15 (1.02–1.29)	0.02
Retransplant vs. first transplant	3.05 (1.77–5.25)	<0.001
NHB donor vs. HB donor	17.3 (8.15–36.9)	<0.001

Table S2: Multivariate risk analysis for delayed graft function.*

* Logistic regression model for the risk of delayed graft function, with aberrant vascular anatomy as extra covariate, added post hoc. SCD denotes standard criteria donation, ECD expanded criteria donation, NHB non-heart beating, and HB heart beating.

† Aberrant vascular anatomy was defined as >1 renal artery of the kidney graft.



No. of values available														
Cold storage-arm	327	329	328	319	322	309	316	282	295	268	266	242	248	278
Machine perfusion-arm	322	324	325	326	316	313	314	296	286	281	273	251	245	274

Median serum creat. (µmol/l)														
Cold storage-arm	592	507	406	327	283	248	221	210	200	195	180	180	177	166
Machine perfusion-arm	557	455	328	269	224	193	182	173	174	168	165	171	168	162

Figure S2: Time course of serum creatinine after transplantation.
Below the x-axis, the sample size available per posttransplant day, and median serum creatinine values per day are indicated. For each recipient, the area under the curve (AUC) was calculated. Missing values (12%) were imputed by means of linear interpolation. The median AUC of recipients in the machine perfusion-arm (1,456; range 385–5,782) was significantly lower than the median AUC of recipients in the cold storage-arm (1,787; range 288–6,500) (P=0.01; Wilcoxon signed rank test).
Each median value is based on all available cases for that group, and includes patients who were dialysis dependent.

Additional non-heart beating donor inclusions

in September 2006, when donor inclusions into the study were nearly complete, the scientific steering committee expected that insufficient non-heart beating donors would be enrolled at trial completion to conduct a meaningful subgroup analysis for this donation type. At the suggestion of the steering committee and with permission of all centers, inclusions of only non-heart beating donors were extended, until a total of 82 were enrolled on 17 August 2007.

Variable	Hazard ratio (95% confidence interval)	P value
Graft failure within 1 year posttransplant†		
Machine perfusion vs. cold storage	0.52 (0.29–0.92)	0.03
NHB donor vs. HB donor	0.87 (0.27–2.81)	0.82
Recipient age (yr)	0.97 (0.95–1.00)	0.02
Duration of pre-transplant dialysis (yr)	1.00 (0.87–1.15)	0.97
Panel-reactive antibody rate (%)	1.01 (0.99–1.03)	0.30
Cold ischemic time (h)	1.04 (0.97–1.10)	0.29
Donor age (yr)	1.05 (1.01–1.10)	0.02
ECD donor vs. SCD donor	1.17 (0.42–3.27)	0.76
HLA mismatches (no.)	1.23 (0.98–1.55)	0.07
Aberrant vascular anatomy‡	1.35 (0.66–2.74)	0.41
Retransplant vs. first transplant	1.73 (0.89–3.36)	0.11

Table S3: Multivariate risk analysis for graft failure.*

* Cox non-proportional hazards model for the risk of graft failure within 1 year posttransplant, with delayed graft function as extra covariate, added post hoc. SCD denotes standard criteria donation, ECD expanded criteria donation, NHB non-heart beating, and HB heart beating.

† Censored upon death with a functioning graft.

‡ Delayed graft function was added to the model as a time dependent covariate.

Variable	Hazard ratio (95% confidence interval)	P value
Graft failure within 1 year posttransplant†		
NHB donor vs. HB donor	0.48 (0.16–1.48)	0.20
Machine perfusion vs. cold storage	0.60 (0.34–1.06)	0.08
Duration of pre-transplant dialysis (yr)	0.96 (0.84–1.10)	0.56
Recipient age (yr)	0.97 (0.95–0.99)	0.005
Panel-reactive antibody level (%)	1.01 (0.99–1.02)	0.54
Cold ischemic time (h)	1.02 (0.95–1.08)	0.61
Donor age (yr)	1.04 (1.00–1.08)	0.03
ECD donor vs. SCD donor	1.10 (0.44–2.72)	0.84
Retransplant vs. first transplant	1.13 (0.58–2.18)	0.72
HLA mismatches (no.)	1.19 (0.96–1.47)	0.12
Delayed graft function‡	1.69 (1.35–2.11)	<0.001

Table S4: Multivariate risk analysis for graft failure.*

* Cox proportional hazards model for the risk of graft failure within 1 year posttransplant, with aberrant vascular anatomy as extra covariate, added post hoc. SCD denotes standard criteria donation, ECD expanded criteria donation, NHB non-heart beating, and HB heart beating.

† Censored upon death with a functioning graft.

‡ Aberrant vascular anatomy was defined as >1 renal artery of the kidney graft.

Fig. S1 and Table S1 in this appendix present a Consort diagram and demographics for the two eras of non-heart beating donor inclusions. Solely for the heart beating/non-heart beating subgroup analysis, these additional inclusions were added to the main set of cases to provide more statistical power. The main set of cases consisted of 336 kidney pairs (672 recipients), of which 42 (84 recipients) came from non-heart beating donors. The extended data set comprised a total of 376 kidney pairs (752 recipients). This total includes the 80 recipients of 40 non-heart beating kidney pairs who were later added to the main set for this analysis only. Therefore, the total number of non-heart beating kidney pairs in the extended data set was 82 (164 recipients). The same logistic regression model for delayed graft function with an interaction term for machine perfusion and non-heart beating donation was applied to the extended data set.

Chapter 6

Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death: a multicenter, randomized, controlled trial

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ABSTRACT

Objective

Hypothermic machine perfusion may improve outcome after transplantation of kidneys donated after cardiac death (DCD), but no sufficiently powered prospective studies have been reported. Because organ shortage has led to an increased use of DCD kidneys, we aimed to compare hypothermic machine perfusion with the current standard of static cold storage preservation.

Methods

Eighty-two kidney pairs from consecutive, controlled DCD donors 16 years or older were included in this randomized controlled trial in Eurotransplant. One kidney was randomly assigned to machine perfusion and the contralateral kidney to static cold storage according to computer-generated lists created by the permuted block method. Kidneys were allocated according to standard rules, with concealment of the preservation method. Primary endpoint was delayed graft function (DGF), defined as dialysis requirement in the first week after transplantation. All 164 recipients were followed until 1 year after transplantation.

Results

Machine perfusion reduced the incidence of DGF from 69.5% to 53.7% (adjusted odds ratio: 0.43; 95% confidence interval 0.20-0.89; P = 0.025). DGF was 4 days shorter in recipients of machine-perfused kidneys (P = 0.082). Machine-perfused kidneys had a higher creatinine clearance up to 1 month after transplantation (P = 0.027). One-year graft and patient survival was similar in both groups (93.9% vs 95.1%).

Conclusions

Hypothermic machine perfusion was associated with a reduced risk of DGF and better early graft function up to 1 month after transplantation. Routine preservation of DCD kidneys by hypothermic machine perfusion is therefore advisable.

INTRODUCTION

Kidney grafts can be preserved by either static cold storage or hypothermic machine perfusion. Static cold storage preserves grafts on melting ice after a cold vascular flush with a preservation solution. Hypothermic machine perfusion preserves the graft by continuous or pulsatile administration of a recirculating cold preservation solution (1–10°C). Optimal preservation of kidney grafts is essential to reduce the risk of delayed graft function (DGF) after transplantation.[31] Indeed, DGF negatively influences long-term graft survival, is associated with a higher risk of acute rejection, and causes increased mortality in older recipients.[31,123-125] DGF inevitably augments postoperative costs because of prolonged hospital stay, the need for dialysis, and additional diagnostic procedures.[126,127]

Currently, because of the persistent donor shortage, kidneys donated after cardiac death (DCD) have become an important additional source of renal allografts in many countries.[128] They have the potential to increase the number of kidney transplantations up to 4.5 times.[128,129] DCD kidneys suffer from a substantially higher incidence of DGF (28%–88% vs 13%–35%), which seriously limits their use, than kidneys donated after brain death (DBD).[40,51,128] This increased rate of DGF is caused by inevitable exposure of these kidneys to renal warm ischemic injury during the period of circulatory arrest. Therefore, optimal preservation of DCD kidneys is crucial to reduce their intrinsically increased risk of DGF and allow a safer and wider use of this potentially large donor source.

Previous studies have suggested that machine perfusion of DCD kidneys results in better early function and improved graft survival than those preserved by static cold storage.[54,130,131] Other studies do not support this conclusion, however, and a comprehensive meta-analysis failed to show a statistically significant risk reduction of DGF in machine-perfused versus static cold-stored DCD kidneys.[105,120,132] Recently, a randomized controlled trial — the Machine Preservation Trial — demonstrated that machine perfusion reduces the risk and duration of DGF compared with static cold storage in kidneys from deceased donors.[48] However, this trial included a majority of DBD donors (87.5%) and was not designed to allow detailed analysis of the effect of machine perfusion on DCD kidneys alone, thereby leaving this critical issue unresolved.

Given this persisting controversy, we conducted a prospectively planned study as a prespecified extension of the Machine Preservation Trial to specifically determine the effect of machine perfusion versus static cold storage on posttransplant outcome of DCD kidneys.

METHODS

Trial enrollment criteria

This prospectively planned analysis assessed all consecutive DCD kidney donors reported in Belgium and the Netherlands during the Machine Preservation Trial. The study was fully integrated in the Eurotransplant system that manages waiting lists and organizes organ allocation in a part of western Europe.[107] The trial included only Maastricht category III (cardiac arrest after withdrawal of treatment) DCD donors 16 years or older and a 5 minute "no-touch" period was always respected.[128] A strictly paired design was maintained, in which both kidneys of 1 donor needed to be transplanted into different recipients. Both kidneys of a pair were excluded from the analysis when 1 or both recipients died within 1 week after transplantation. To allow complete integration within Eurotransplant, to reflect current practice, and to ensure the participation of all transplant centers, current standard center protocols were not changed. Informed consent from recipients was not required, as kidneys were randomized before organ allocation. Ethical approval was obtained from the Eurotransplant Ethical Advisory Committee, the Kidney Advisory Committee, and ethics review boards in each trial region.

Randomization

Whenever a potential kidney donor meeting the inclusion criteria was reported, the Eurotransplant duty desk officer randomly assigned 1 kidney to machine perfusion and the contralateral kidney to static cold storage. Randomization lists were computer generated by the permuted block method. We used regional lists to avoid imbalances caused by small differences in allocation algorithms. When a reliable connection to the perfusion machine was impaired by a too small aortic patch or too many renal arteries, randomization for this kidney pair was changed and preservation methods were switched. Kidneys were allocated according to standard Eurotransplant allocation rules, without revealing the preservation method at organ offer. The recipient's surgical team was unblinded at the time of transplantation.

Preservation methods

Hypothermic machine perfusion was performed with LifePort Kidney Transporter machines (Organ Recovery Systems, Itasca, IL). For the purpose of the study, a trained perfusionist attended each donor procedure to guarantee availability and correct use of the machines. Immediately after organ recovery, the donor surgeon, assisted by the perfusionist, connected the kidney randomized to machine perfusion to the perfusion machine. A pulsatile flow with Kidney Preservation Solution-1 (KPS-1) (1–8°C) was maintained until transplantation.[108] Systolic perfusion pressure was fixed at 30 mm Hg. Next, the machine-perfused kidney was transported to the recipient hospital without any monitoring. Flow readings and intravascular resistance were concealed to the transplantation team. As a result, the decision to accept or reject a kidney could not be biased by these parameters.

Kidneys randomized to static cold storage were flushed and preserved according to the established Eurotransplant routine, using either University of Wisconsin solution (UW) or histidine-tryptophan-ketoglutarate (HTK) according to the center-specific practice. Organs were submerged in the preservation solution and stored on melting ice until transplantation.

Follow-up

No changes to center-specific patient follow-up protocols were made. Eurotransplant established a secure online database in which follow-up data could be provided by participating transplantation centers. To ensure maximal data completeness, recipient centers were financially compensated for providing follow-up data. No relevant irregularities were found during an external audit of a random sample of 10% of all patient follow-up data.

Study endpoints

The primary endpoint was DGF, defined as the need for dialysis in the first week after transplantation. As a secondary endpoint, early graft function was assessed in a more refined, objective way as functional DGF, which was defined as the absence of a decrease in serum creatinine level by a minimum of 10% per day during 3 consecutive days in the first postoperative week, not including patients in whom acute rejection, calcineurin inhibitor toxicity, or both, developed within the first week.[109] Other secondary endpoints were as follows: duration of DGF, primary nonfunction (PNF), biopsy-proven acute rejection, calcineurin inhibitor toxicity, serum creatinine values, creatinine clearance, length of recipients' hospital stay, and patient and graft survival up to 1 year after transplantation. Data on graft survival were censored at the time of death in patients who died with a functioning graft.

Statistical methods

All data analyses were performed using SPSS, SAS, and R software. Two-sided P values 0.05 or less indicated statistical significance. The study was powered to detect a reduction in DGF due to machine perfusion of at least 20%, based on a presumed rate of DGF of 70% in the cold storage group. A minimum of 80 kidney pairs were required to obtain a statistical power of 0.8, assuming a univariate 1-sided type I error of 0.05; this is equivalent to the required sample size for a multivariate logistic regression with a 2-sided type I error and similar power.[110] The influence of machine perfusion compared with static cold storage on the risk of DGF was examined by a logistic regression model.[110] Covariate selection was based on relevant literature and prespecified in the protocol before the trial started.[112,113] To better reflect the paired study design, all covariates were entered in the analysis with a built-in normal-gamma frailty term for the donor. Demographic variables were analyzed by the Fisher exact test or the Mann-Whitney test. We applied the McNemar test or the Wilcoxon signed-rank test to evaluate univariate differences in endpoint variables between the 2 groups. Assessment of graft and patient survival was done by the Kaplan-Meier method and differences between survival curves were determined by log-rank tests. Endpoint interim analyses were not

performed, but confidential safety analyses comparing reported rates of adverse events in the 2 study groups were conducted at regular intervals by the trial safety board.

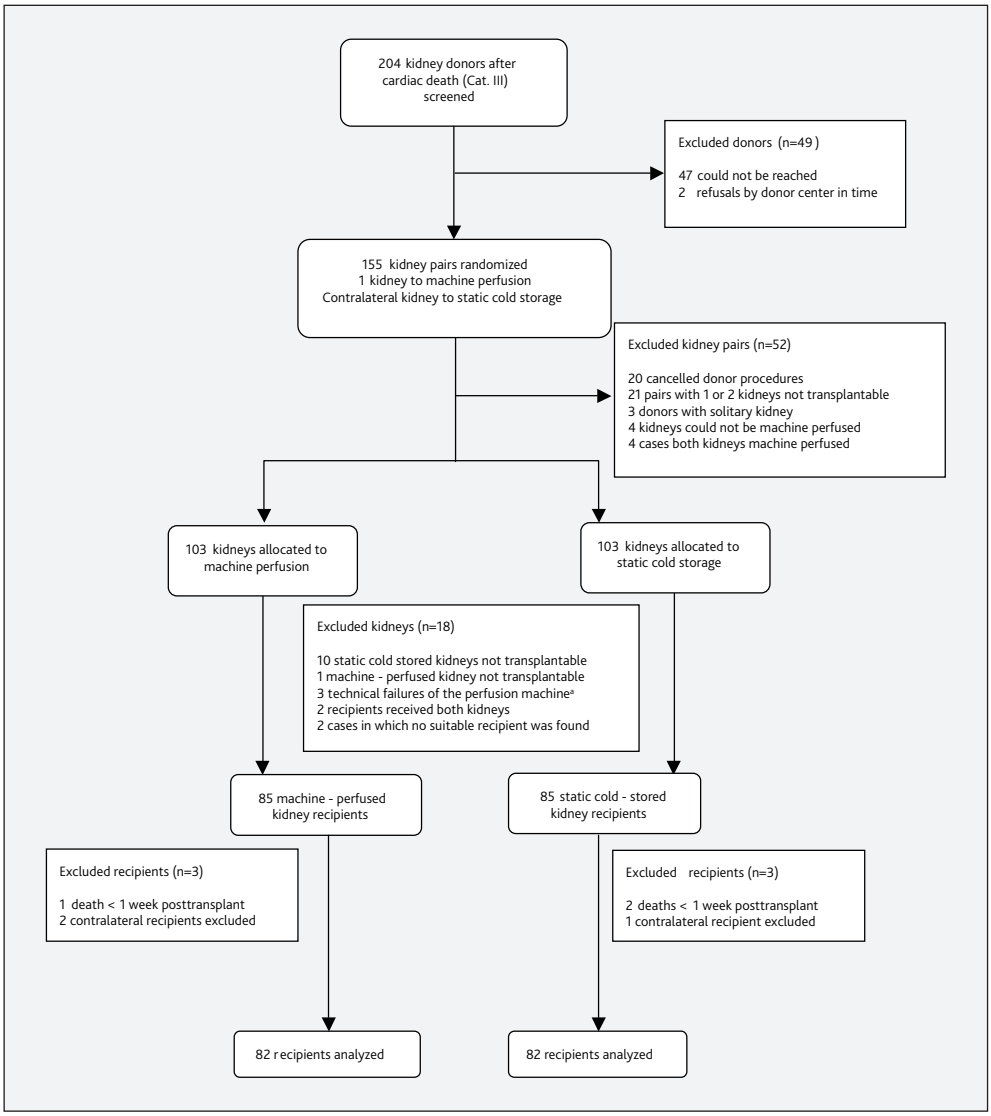

Figure 1: Consort diagram showing enrollment and randomization of donors kidney pairs in the trial.
[a] Technical machine-related problems caused the machine to switch to the "fail safe" mode and led to cold storage of the kidney inside the machine. These kidneys remained suitable for transplantation but were excluded from analysis in the present study. Because the machine perfusion pump is pressure controlled, the "fail safe" mode is activated when a risk of possible barotrauma is detected. This occurred in 3 cases: (1) a sudden change in surrounding pressure during transport misguided the software, (2) a high resistance alarm, and (3) leakage of the perfusion fluid.

Role of funding source

An independent scientific steering committee comprising clinicians and scientists from each trial region was responsible for the design, conduct, data analysis, and manuscript preparation for this study. The sponsor was not involved in the study design, follow-up data acquisition, data analyses, or writing of the manuscript. During the course of this trial, the sponsor provided the trial regions with machine perfusion devices and disposables free of charge and operated a 24-hour helpline that could be consulted by perfusionists in case of perfusion device–related technical issues.

RESULTS

Donors of DCD kidney pairs were enrolled into the present study in 2 phases. In the first phase (November 1, 2005, to October 31, 2006), enrollment was conducted as part of the larger Machine Preservation Trial.[17] Near the end of donor enrollment in this main trial, the steering committee anticipated that insufficient DCD kidney pairs would be included to perform relevant analyses for the prespecified DCD subgroup. Inclusion of DCD donors therefore was continued in a second phase (November 1, 2006, to August 17, 2007) adhering to the protocol of the Machine Preservation Trial. The flow diagram (Fig. 1) shows enrollment and randomization of kidney pairs for the present study. Two hundred four potential DCD kidney donors were assessed for inclusion, 103 kidney pairs were included, and data from 82 recipients in each study group were analyzed. In 9 cases, the connection of the kidney randomized to machine perfusion was unreliable because of aberrant vascular anatomy and therefore preservation methods of both kidneys were switched. Vascular anomalies, however, did not significantly increase the risk of DGF (data not shown, P = 0.064).

Study group characteristics

Table 1 shows the characteristics of kidney donors and recipients. Eighty-two recipients were included in each study group. There were no significant differences between the groups with respect to donor and recipient age, duration of pretransplant dialysis, number of previous transplants, panel reactive antibodies, cold ischemic time, flush solution, induction therapy, and maintenance immunosuppression regimens.

Primary endpoint

Forty-four recipients in the machine perfusion group and 57 recipients in the static cold storage group developed DGF (53.7% vs 69.5%; P = 0.007) (Table 2). Multivariate analysis (Table 3) showed a decreased probability of developing DGF in machine-perfused versus static cold-stored DCD kidneys (adjusted odds ratio: 0.43; 95% confidence interval: 0.20–0.89; P = 0.025). Other significant risk factors for DGF were donor and recipient age and warm and cold ischemic times.

Variable	Machine perfusion group (N = 82)	Static cold storage group (N = 82)	P
Donor characteristics			
Age,* median (range), yr	43 (17–67)		
Warm ischemic time,† median (range), min	16 (6–38)		
n < 10 min	21		
n = 10–19 min	40		
n = 20–29 min	18		
n ≥ 30 min	6		
Flush solution: HTK/UW/other	62/18/2		
Cold ischemic time‡			
Median (range)	15.0 (4.3–28.9)	15.9 (8.6–46.6)	0.70
Mean (25th–75th percentile)	16.6 (14.2–19.8)	17.3 (13.9–19.7)	0.41
n > 24 h	4	6	
Recipient characteristics			
Age, median (range), yr	49 (24–73)	52 (24–77)	0.81
Duration pretransplant dialysis, median (range), yr	4.2 (1.0–17.5)	4.0 (0.4–10.7)	0.48
Previous transplants, n			0.82
First transplant	71	70	
Retransplant	11	12	
Panel reactive antibodies, %			0.73
n = 0–5	71	71	
n = 6–84	11	10	
n ≥ 85	0	1	
No mismatches at HLA-A, B, DR loci, %	2.4	3.7	0.50
Immunosuppression,n			
Antithymocyte globulin	12	13	0.71
Interleukin 2 receptor antagonist	37	31	0.34
Azathioprine	1	1	0.61
Cyclosporin A	37	31	0.34
Tacrolimus	43	52	0.25
Corticosteroids	81	81	1.00
Mycophenolate mofetil	69	76	0.14

Table 1: Characteristics of donors and recipients

* Fourteen DCD donors also fulfilled the criteria for expanded criteria donors as determined by the United Network for Organ Sharing; 8 were older than 60 years.[33]

† Warm ischemic time: time from circulatory arrest until the start of cold perfusion.

‡ Cold ischemic time: time from start cold perfusion until the start of kidney implantation.

HLA indicates human leukocyte antigen.

Variable	Machine perfusion group (N = 82)	Static cold storage group (N = 82)	P
Delayed graft function*			
Incidence, n (%)	44 (53.7)	57 (69.5)	0.007
Duration			0.021
<7d	12	6	
≥7 d	32	51	
Median duration, d	9 (1–48)	13 (2–43)	0.082
Functional delayed graft function,† n (%)	16 (19.5)	42 (51.2)	<0.0001
Primary nonfunction, ‡ n (%)	2 (2.4)	2 (2.4)	1.00
Acute rejection within 14 d, n (%)	6 (7.3)	10 (12.2)	0.28
Calcineurin inhibitor toxicity, n (%)	13 (15.9)	10 (12.2)	0.34
Serum creatinine value, median (range), mg/dL			
14 d posttransplant	4.1 (0.9–11.2)	5.1 (1.0–11.3)	0.001
1 mo posttransplant	1.7 (0.9–7.1)	2.1 (0.7–9.9)	0.017
3 mo posttransplant	1.5 (0.8–5.4)	1.5 (0.6–8.3)	0.021
Creatinine clearance, median (range), mL/min			
14 d posttransplant	23 (3–98)	13 (0–160)	<0.0001
1 mo posttransplant	46 (10–98)	35 (1–113)	0.027
3 mo posttransplant	57 (11–128)	49 (11–104)	0.117
Length of recipient hospital stay, median (range), d	17 (7–392)	19 (8–65)	0.24
Allograft survival at,n (%)			
3 mo	79 (96.3)	79 (96.3)	
1 yr	77 (93.9)	78 (95.1)	
Recipient survival at,n(%)			
3 mo	81 (98.8)	82 (100)	
1 yr	79 (96.3)	80 (97.6)	

Table 2: Univariate analysis of trial endpoints
* Delayed graft function: need for dialysis in the first week after transplantation.
† Functional delayed graft function: lack of ≥10% serum creatinine decrease per day during 3 consecutive days in the first week after transplantation.[20] Recipients developing acute rejection or calcineurin inhibitor toxicity within the first week were excluded from this category.
‡ Primary nonfunction: permanent lack of graft function.

Secondary endpoints

Table 2 shows the univariate analysis of all secondary endpoints. Functional DGF occurred in 16 recipients in the machine perfusion group versus 42 recipients in the static cold storage group (19.5% vs 51.2%; P < 0.0001). The median duration of DGF in the machine perfusion group was 4 days shorter than in the static cold storage group, but this difference did not reach statistical significance (9 days vs 13 days; P = 0.082). However, DGF was more likely to be shorter than 7 days in a machine-perfused kidney than in a static cold-stored kidney.

There were no differences in the median length of recipients' hospital stay. PNF occurred in only 2 cases in each study group. Creatinine clearance was significantly higher in the machine perfusion group until 1 month after transplantation. At 1 year follow-up, 3 patients in the machine perfusion group and 2 patients in the static cold storage group had died. Graft survival at 1 year follow-up was similar in both groups (93.9% vs 95.1%) (Fig. 2).

Variable	Adjusted odds ratio (95% confidence Interval)	P
Machine perfusion vs static cold storage	0.43 (0.20–0.89)	0.025
Donor age, yr	1.04 (1.01–1.08)	0.008
Recipient age, yr	1.04 (1.00–1.08)	0.028
Retransplant vs first transplant	0.77 (0.39–1.54)	0.46
Panel reactive antibody level, %	2.97 (0.90–9.87)	0.075
HLA mismatches, n	1.28 (0.87–1.88)	0.21
Duration of pretransplantation dialysis, d	1.01 (0.88–1.27)	0.92
Cold ischemic time, h	1.10 (1.01–1.21)	0.039
Warm ischemic time (10 min)*	3.40 (1.87–6.17)	<0.0001

Table 3: Multivariate analysis of the risk of delayed graft function
* Warm ischemic time: time from circulatory arrest until the start of cold perfusion. Warm ischemic time was grouped into 10-minute intervals and a warm ischemic time of less than 10 minutes was used as the baseline.
HLA indicates human leukocyte antigen.

Complications

No vascular complications of the graft (arterial thrombosis, dissection, etc) were seen in either group. Cardiovascular, gastrointestinal, infectious, metabolic, urinary, and technical complications were comparable between the groups and within reported ranges in the literature (data not shown).

DISCUSSION

This multicenter randomized, controlled trial demonstrated the superiority of machine perfusion over static cold storage for the preservation of DCD kidneys. This is an important finding, as DGF after kidney transplantation adversely influences outcome, causes morbidity and even mortality in older recipients, and leads to additional costs.[1–6] DCD kidneys are currently accepted by many transplant centers as an additional donor source and the potential of DCD kidneys is large. Because DCD kidneys are intrinsically more prone to developing DGF, decreasing the incidence of DGF by machine perfusion will be particularly beneficial for recipients of this type of kidney graft.[40,51,128]

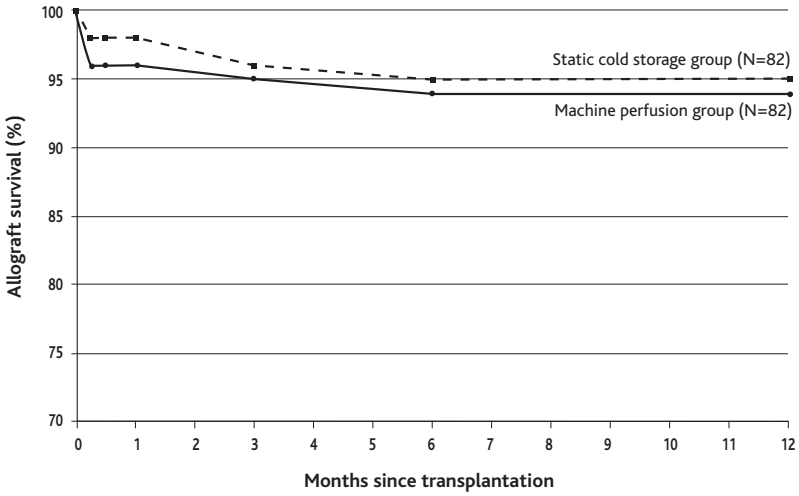

Figure 2: Death censored allograft survival at 1 year after transplantation. Graft survival in the machine perfusion versus the static cold storage group was similar (94% vs 95%) (log-rank test of equality: P = 0.7).

In the multivariate analysis, machine perfusion clearly reduced the risk of DGF. Furthermore, DGF was more likely to be short lasting (<7 days) in machine-perfused kidneys than in static cold-stored kidneys. We also explored the impact of machine perfusion on functional DGF, which is a more refined surrogate marker for early kidney graft function than DGF defined as dialysis requirement in the first postoperative week.[109] We found that the incidence of functional DGF was strongly reduced by machine perfusion, even more than the incidence of DGF. Hence, the protective effect of machine perfusion shown in our study may be underestimated when using only the traditional definition of DGF as an outcome measure. However, we selected the traditional definition of DGF as the primary endpoint to allow for comparison of the results in the present analysis with those from previous studies. Our observation that creatinine clearance in recipients of machine-perfused kidneys was higher early after transplantation shows that actual early kidney function is also superior after machine perfusion.

Our study confirmed that donor age and cold ischemic time are independent risk factors for DGF in DCD kidneys, even though cold ischemic times were relatively short in both groups.[112,113] Cold ischemic time was slightly but not significantly longer in the static cold-stored group. However, with a previously reported odds ratio of 1.23 of DGF for every 6-hour increase in cold ischemic time,[133] it is unlikely that these additional 54 minutes of cold ischemia caused a major bias of the primary endpoint. Moreover, the study revealed that the duration of warm ischemia is a more important independent additional risk factor for DGF.

Even though DGF is a risk factor for graft failure after kidney transplantation and machine perfusion significantly reduced the risk for DGF, our study did not show improvement in 1-year graft survival of machine-perfused versus static cold-stored kidneys.[31,123,125] We cannot exclude that the young donor age in our cohort in part masked an advantage of machine perfusion on graft survival. Nevertheless, this finding is in line with an increasing number of reports showing similar medium-term graft survival for DCD and DBD kidneys despite higher rates of DGF in DCD kidneys.[51,128,134] DGF does not influence graft survival after DCD kidney transplantation in the same way as it does after DBD kidney transplantation. This could be explained by a possibly different nature of DGF in DCD versus DBD kidneys. The metabolic, hemodynamic, hormonal, and inflammatory changes that occur after brain death and during donor management, but not after cardiac death, may impair kidney function more and could have more long-term impact than warm ischemic injury alone.[135,136]

The present study yielded a few surprising results. First, despite the significant reduction of DGF by machine perfusion, the incidence of PNF was not reduced. A PNF incidence of only 2.4% may seem surprisingly low. The exclusion of uncontrolled (Maastricht category I and II) DCD donors who are more prone to PNF and the relatively short median warm and cold ischemic times in our donor population may account for the low rate of PNF. However, when compared with reported PNF incidences in other series of controlled DCD kidney transplantations (0%–17%) and the previously conducted main trial (1/42), the observed incidence of PNF was not exceptionally low.[48,120,130,131,137,138] Nevertheless, it is likely that the overall incidence of PNF was too low to detect an effect of machine perfusion. Second, hospital stay is usually longer in recipients of DCD versus DBD kidneys because of the increased rate of DGF in the former group.[51] Despite reduced duration and severity of DGF, our study showed no significant reduction in hospital stay for recipients of machine-perfused kidneys. This unexpected observation may, at least in part, be explained by the fact that the trial was conducted in Eurotransplant countries. Healthcare systems with greater pressure to limit the use of resources will have a tendency toward shorter hospital stay.[139,140] We believe that in countries with such a healthcare system, the reduction in DGF seen in our trial might be paralleled by a significant reduction in hospital stay. This observation also reflects the relative unreliability of hospital stay as a valid outcome parameter, as suggested by other studies.

To date, no definitive evidence of the superiority of machine perfusion over cold storage for the preservation of DCD kidneys has been available. Although an advantage of machine perfusion has been suggested, all previous studies were relatively small in size compared with the present prospective trial.[54,105,130,131] The effect of machine perfusion on DCD kidney preservation was recently studied in the United Kingdom. A randomized controlled trial with sequential analysis suggested that machine perfusion of DCD kidneys does not decrease DGF.[138] Differences in study design may account for this discrepancy. The present trial was not only larger but also fully integrated into Eurotransplant. Kidneys were allocated strictly

and solely according to standard Eurotransplant rules, and recipient centers were blinded to the preservation method at the time of organ offer. Furthermore, all kidneys were perfused immediately after retrieval until transplantation, which was not necessarily the case in other studies. The need to perfuse kidneys immediately after retrieval to benefit fully from the "perfusion effect" needs to be investigated further, as this practice has important logistic consequences.

The present study has some limitations. The strictly paired design of the trial, and the necessity to randomize kidney pairs immediately after the report of a potential donor, accounts for the large number of exclusions. First, donor kidney pairs, of which 1 kidney was not transplanted, were excluded, possibly leaving kidneys with a higher risk of DGF out of the study. Second, less hemodynamically stable donors, in whom organ recovery had to be performed as an emergency procedure, could not always be reached in time. Another possible limitation is the difference in preservation solutions in both groups. Only 1 pharmaceutical formulation of machine preservation solution is Food and Drug Administration approved; therefore, machine-perfused kidneys were preserved with KPS-1. Static cold-stored kidneys were preserved in HTK (75.6%) or UW (22.0%), and although UW is still the gold standard for cold storage of kidneys, analysis of the United Network of Organ Sharing data showed that HTK preservation has no effect on DGF compared with UW.[141]

In conclusion, this international randomized, controlled trial showed that hypothermic machine perfusion of DCD kidneys reduced the risk of DGF and improved graft function until 1 month after transplantation. When DGF occurred, it was of a shorter duration and less severe. We therefore suggest that machine perfusion should be routinely used for the preservation of DCD kidneys. Apart from being beneficial to the individual patient, these protective effects of machine perfusion might result in a substantial reduction of DGF-related costs. The cost-effectiveness of hypothermic machine perfusion however remains to be investigated.

Chapter 7

Machine perfusion versus cold storage for preservation of kidneys from expanded criteria donors after brain death

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ABSTRACT

The purpose of this study was to analyze the possible effects of machine perfusion (MP) versus cold storage (CS) on delayed graft function (DGF) and early graft survival in expanded criteria donor kidneys (ECD). As part of the previously reported international randomized controlled trial 91 consecutive heartbeating deceased ECDs – defined according to the United Network of Organ Sharing definition – were included in the study. From each donor one kidney was randomized to MP and the contralateral kidney to CS. All recipients were followed for 1 year. The primary endpoint was DGF. Secondary endpoints included primary nonfunction and graft survival. DGF occurred in 27 patients in the CS group (29.7%) and in 20 patients in the MP group (22%). Using the logistic regression model MP significantly reduced the risk of DGF compared with CS (OR 0.460, P = 0.047). The incidence of nonfunction in the CS group (12%) was four times higher than in the MP group (3%) (P = 0.04). One-year graft survival was significantly higher in machine perfused kidneys compared with cold stored kidneys (92.3% vs. 80.2%, P = 0.02). In the present study, MP preservation clearly reduced the risk of DGF and improved 1-year graft survival and function in ECD kidneys.

INTRODUCTION

As a result of persistent donor organ shortage, kidneys from expanded criteria donors (ECDs) are nowadays accepted by many centers and successfully transplanted, thus shortening waiting times.[12,142,143] Unfortunately, kidneys from ECDs appear to have a higher rate of delayed graft function (DGF) and a more complicated postoperative course, resulting in an inferior long-term graft survival overall.[12,111-113,142] Although the use of kidneys from ECDs has an overall risk for graft failure of 1.7, it has also been shown that transplantation of these kidneys has a significant survival benefit compared with dialysis treatment.[144]

To enhance the outcome of using ECD kidneys, it is important to analyze the risk factors, including the role of the preservation method. A recently published systematic review suggests that hypothermic machine perfusion (MP) might be superior compared with simple cold storage (CS), reducing the relative risk of DGF by up to 20% and increasing 10-year graft survival by 6%.[105,106] However, this evidence is based on studies that were limited by an uncontrolled patient selection, small patient numbers, the use of different and sometimes out-of-date preservation solutions, nonstandardized pumping modes and times, as well as inconsistent application of currently available pump technology.

We recently reported the overall results of an international multi-center randomized trial comparing MP versus CS in unselected consecutive donors ≥16 years of age, demonstrating the safety of MP and a significant reduction in both DGF and 1-year graft loss.[48] As this effect might be even more pronounced or clinically relevant in ECD kidneys,[12,145] the purpose of this study was to provide an analysis of the possible effects of MP versus CS on DGF and early graft survival in ECD kidneys.

METHODS

As part of the previously reported multi-center randomized trial[48] all consecutively retrieved kidney pairs from heart-beating deceased ECDs in the Netherlands, Belgium, and the federal state of North Rhine-Westphalia in Germany between November 1, 2005 and October 31, 2006 were eligible for randomization. ECDs were defined according to the United Network of Organ Sharing (UNOS) definition,[12,143] which includes: donor age ≥60 years or 50–60 years with at least two of the following criteria: history of hypertension, cerebrovascular cause of death and serum creatinine 132 µmol/l (1.5 mg/dl) prior to retrieval.

Donors were only included in the study for analysis if both organs were transplanted into two different recipients. Donors accepted for combined organ transplantation (e.g., liver–kidney transplantation) by the recipient center were excluded from the trial.

Recipient centers were blinded to the method of preservation (MP or CS) at the time of acceptance of the kidney for a specific recipient.

The study protocol was approved by ethics committees in each trial region. The study

was sponsored by the Deutsche Forschungsgemeinschaft (DFG TR 811/1-1) and by Organ Recovery Systems (Itasca, IL, USA).

Randomization and logistics

From each donor, one kidney was randomized to MP and the contralateral kidney to CS. The randomization process and logistic management have been described in an earlier publication.[48]

Preservation methods

All kidneys underwent in situ vascular washout with cold preservation solution (histidine–tryptophan–ketoglutarate or University of Wisconsin solution). Kidneys assigned to hypothermic MP were connected to a LifePort Kidney Transporter® (Organ Recovery Systems) shortly after procurement and machine perfused until transplantation. A pulsatile flow of machine preservation solution (Kidney Preservation Solution-1®; Organ Recovery Systems, Itasca, IL, USA) at 1–8 °C and a fixed systolic perfusion pressure of 30 mmHg were maintained. The transplant team was blinded to MP intravascular resistance and flow data. Kidneys assigned to CS were submerged in preservation solution and stored on melting ice.

Endpoints and data collection

The primary endpoint of this ECD study was DGF defined as the need for dialysis during the first week posttransplant. Secondary endpoints were: functional delayed graft function (f-DGF), which is defined as the absence of a decrease in serum creatinine levels of at least 10% per day for at least three consecutive days in the first week after transplantation;[109] duration of DGF; primary nonfunction (PNF) of the transplanted kidney; serum creatinine levels at 1–14 days and 1, 3 and 12 months; creatinine clearance at 1 and 2 weeks and 1, 3 and 12 months; biopsy-proven acute rejection and calcineurin inhibitor toxicity within the first 2 weeks; recipient hospital stay length; graft and patient survival; and the number of biopsy-proven rejection and calcineurin inhibitor toxicity episodes up to 1 year posttransplant.

In addition, standard donor and recipient data and the type of induction immunosuppression therapy were recorded. Follow-up data were collected in a secure online database hosted by Eurotransplant and were provided by each of the 60 participating transplant centers.

Statistics

The analysis was powered to detect a reduction in DGF of at least 20% based on the assumption of a 40% incidence of DGF in recipients with kidneys preserved by CS. With a power of 0.8 and a type I error of 0.05, the required sample size was 82 pairs of ECD kidneys. The primary analysis of the DGF endpoint consisted of a logistic regression model with the covariates shown in Table 3.

Secondary endpoint variables were assessed for univariate differences between groups by the McNemar or the Wilcoxon signed rank test. Differences between survival curves were

determined by the log rank test. A Cox proportional hazards model was applied to examine which variables significantly influenced the risk of graft failure. All P-values are two-sided and not adjusted for multiple testing. Analyses were conducted using SPSS (IBM Corporation, Somer, NY, USA), SAS (SAS Institute Inc., Cary, NC, USA) and R software packages (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Between November 1, 2005 and October 31, 2006, 336 out of 654 deceased donors 16 years of age and older were included in the overall study. Of these 654 donors, 200 were ECDs. There were 109 ECDs who were not studied, thus 91 donors were included in the subgroup analysis. The reasons for exclusion are described in Table 1. The main reason was that one or both kidneys were not transplantable (42/109). Preservation methods were switched in five donors. In two cases this was attributable to aberrant vascular anatomy, whereas in three cases no reason could be found.

Reason for exclusion	N
Reported after procurement	1
Could not be reached in time	8
Donor center refusal	0
Donor family refusal	0
Donor procedure canceled	5
One or both kidneys not transplantable	42
Combined organ offer	7
Other reasons	32
Kidney rejected at transplant center	4
Technical failure MP	2
Not assessed by mistake	0
Unknown	8

Table 1: Reasons for exclusion of donors. Potential donors N = 200, donors included N = 91, reasons for excluding N = 109, MP, machine perfusion.

Donor and recipient characteristics are summarized in Table 2. The median donor age was 66 years (50–81 years) and the median recipient age was 65 years in both groups. There were no significant differences between the two groups concerning relevant baseline characteristics.

Further subset analysis showed no differences concerning median cold ischemia time between MP and CS for donors older than 65 years (9 h vs. 10 h, P = 0.61) or the subset of transplants with more than three HLA mismatches (10 h vs. 10 h, P = 0.92).

	MP arm	CS arm	P value
Donor characteristics			
Age (years)	66 (50–81)		
Sex (M/F)	49/42		
BMI	27 (21–42)		
Duration of ICU stay (days)	2.5 (0.1–17)		
Serum creatinine (µmol/l)			
mean	96		
max	310		
median (range)	86 (50–310)		
(Nor)adrenalin (Y/N/unknown)	72/19/0		
Preservation solution			
UW	50		
HTK	40		
other	1		
Recipient characteristics			
Age (years)	65 (20–79)	65 (32–79)	0.75
Sex (M/F)	55/36	57/34	0.88
Pre-Tx dialysis duration (days)	1728 (149–3,866)	1728 (137–5,154)	0.68
Previous transplants (0/1/2/3)	69/19/3/0	64/19/6/2	0.29
Current PRA (0-5/6-84/85+%)	85/5/1	81/9/1	0.43
HLA mismatches (% with 0)	12.1	8.8	0.63
Cold ischemic time (hours)	13 (3–23)	13 (4–29)	0.97
Endpoints			
DGF (Y/N)	20/71 (22.0%)	27/64 (29.7%)	0.27
Duration of DGF (days)	14 (3–31)	15 (4–41)	0.45
Duration of DGF <7 days (Y/N)	5/15	4/23	0.22
f-DGF (Y/N)	15/57 (20.8%)	22/52 (29.7%)	0.31
PNF (Y/N)	3/88 (3%)	11/80 (12%)	0.04
CNI intoxicity (Y/N/unknown)	5/78/8	3/81/7	0.63
Acute rejection (Y/N/unknown)	17/64/10	16/67/8	0.98
Creatinine clearance at 1 year (mean±SD, ml/min)	78±41	69±48	0.01

Table 2: Donor and recipient characteristics and results of the univariate analyses. If not indicated otherwise, values for continuous variables represent median (range).

In this ECD subgroup, DGF occurred in 27 patients in the CS group (29.7%) and in 20 patients in the MP group (22%). This difference was not statistically significant in the univariate analysis (P = 0.27) (Table 2). The analysis using the logistic regression model showed that MP significantly

reduced the risk of DGF compared with CS (adjusted odds ratio 0.460, P = 0.047) (Table 3). The number of kidney pairs from the same donor for which both kidneys developed DGF after transplantation was nine.

There was no significant difference in the incidence of DGF in the ECD subgroup compared with the main data set[48] in neither machine perfused kidneys (22% vs. 20.8%) nor in cold stored kidneys (29.7% vs. 26.5%). Further significant factors affecting the risk for DGF were cold ischemia time, duration of pretransplant dialysis, and whether it was a retransplant versus a first transplant.

Secondary endpoints

The incidence of PNF in the CS group (12%) was four times higher than in the MP group (3%) (P = 0.04). Of the cold stored kidneys with PNF in the main dataset, 68% were from ECDs; however, only 42.5% of machine perfused kidneys with PNF came from ECDs (P = 0.52). The PNF in cold stored ECD kidneys was significantly more frequent than in the whole group of cold stored kidneys (P = 0.025), whereas there was no difference in the occurrence of PNF in the machine perfused kidneys when ECDs were compared with all donors. The incidence of f-DGF was 29.7% after CS and 20.8% after MP (P = 0.31) (Table 2).

There were no significant differences between the two groups concerning creatinine clearance up to 3 months, daily creatinine values up to day 14, the incidence of biopsy-proven calcineurin inhibitor toxicity, acute rejection episodes, and the length of hospital stay. Creatinine clearance after 1 year was significantly higher in the MP group compared with the CS group (78 ± 41 ml/min vs. 69 ± 48 ml/min, P = 0.01) (Table 2).

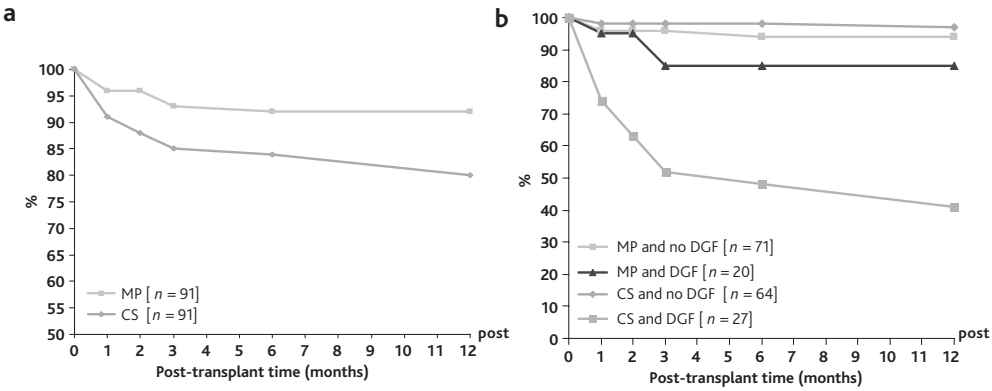

Figure 1: (a) Posttransplant graft survival rates. All consecutive renal transplants from expanded criteria donors after brain death, N=182. Logrank test of equality MP vs. CS p=0.02. **(b)** Posttransplant graft survival rates. All consecutive renal transplants from expanded criteria donors after brain death, N=182. Logrank test of equality: within CS group DGF vs. no DGF P<0.0001; within MP group DGF vs. no DGF P=0.164; within no DGF group MP vs. CS P=0.48; within DGF group MP vs. CS P=0.003.

Patient and graft survival

No patient deaths occurred in the first 14 days after transplantation. Patient survival after 1 year was 93.4% in the MP group and 96.7% in the CS group (P = 0.30). One-year death censored graft survival was significantly higher in machine perfused kidneys compared with cold stored kidneys (92.3% vs. 80.2%, P = 0.02) (Fig. 1a). This difference was even more pronounced if DGF had occurred. Although in the MP group there was a difference of nearly 10% for 1-year graft survival if DGF occurred compared with kidneys with immediate function, this difference was not statistically significant (94% vs. 85%, P = 0.164). In the CS group, graft survival was impressively reduced if DGF occurred (41% vs. 97%, P < 0.0001). If only recipients of grafts that developed DGF were analyzed, there was a significant difference in 1-year graft survival between machine perfused kidneys and cold stored kidneys (85% vs. 41%, P = 0.003) (Fig. 1b).

Cox regression analysis showed that MP significantly reduced the risk of graft failure in the first year with a hazard ratio of 0.353 (P = 0.022) (Table 4). As a relevant defining factor for ECDs, donor age had no significant influence on DGF in this analysis. However, even in this older group of donors, it did significantly influence 1-year graft survival (hazard ratio 1.103, P= 0.016).

Covariate	Odds ratio (95% CI)	P-value
Treatment arm MP vs. CS	0.460 (0.213–0.989)	0.047
CIT (hours)	1.151 (1.057–1.254)	0.001
Number of HLA mismatches	1.905 (0.454–8.000)	0.379
Most recent PRA (%)	1.004 (0.980–1.029)	0.742
Recipient age (years)	1.586 (0.569–4.424)	0.378
Donor age (years)	1.036 (0.957–1.122)	0.385
First vs. re-transplant	2.307 (1.257–4.234)	0.007
Pre-Tx dialysis duration (days)	1.001 (1.000–1.001)	0.021

Table 3: Logistic regression model for the risk of DGF.

DISCUSSION

In the context of this randomized trial[48] we have now focused on the effect of MP in kidneys from ECDs. This effect was even more pronounced than in the overall study, with an odds ratio (OR) of 0.46 for the risk of developing DGF (overall OR of 0.57). Nevertheless, direct comparison of the treatment effects on DGF between expanded criteria donation and standard criteria donation that also included deceased donation after cardiac death showed no significant difference.

It is interesting to see that in this study, the incidence of DGF in ECD kidneys is only slightly higher than in the main data set, irrespective of the preservation method. The incidence of DGF found in this trial is clearly lower than that reported in previous studies using ECD.[121,146] One explanation for this might be the relatively short cold ischemic times in this study.

The hazard ratio for graft failure was also more reduced for ECDs with a value of 0.35 than in the overall study with 0.52. The number of recipients receiving an ECD kidney with PNF was fourfold higher in the CS group compared with the MP group. Such early graft failure, in addition to subsequent graft failures, puts a severe burden on patients and waiting lists for kidney transplantation. The effect we observed was much stronger than described in a recent meta-analysis.[105]

Covariate	Hazard ratio (95% CI)	P-value
Treatment arm MP vs. CS	0.353 (0.145 - 0.862)	0.022
CIT (hours)	1.082 (0.994- 1.179)	0.068
Number of HLA mismatches	4.070 (0.484- 34.208)	0.196
Most recent PRA (%)	1.006 (0.983- 1.030)	0.600
Recipient age (years)	0.629 (0.219- 1.805)	0.388
Donor age (years)	1.103 (1.018-1.195)	0.016
First vs. re-transplant	0.938 (0.480 -1.832)	0.851
Pre-Tx dialysis duration (days)	1.000 (1.000-1.001)	0.495

Table 4: Cox proportional hazards model for the risk of graft failure within 1 year posttransplant.

For ECDs, we also show for the first time that at 1 year posttransplant, the function of the surviving grafts was better if the kidney was preserved by MP compared with CS. These results differ from retrospective studies as these studies show only short term beneficial effects of MP with a reduction of DGF but no improvement in graft survival.[147-149]

Although donor age is already part of the ECD definition, it was the only significant predictive factor in the Cox proportional hazard model for graft survival after 1 year, apart from the treatment modality MP versus CS (Table 4).

The effect of MP on the reduction in serum creatinine levels in the first 14 days compared with cold stored kidneys could not be demonstrated in the ECD group, although, this was shown in the main data set. This is probably because of the smaller sample size of the present study.

f-DGF was chosen instead of the creatinine reduction ratio (CRR) since the CRR only takes into account days 1 and 2 posttransplant. f-DGF has a scope of 7 days after transplantation and has also been validated by Boom *et al*.[109] The scope of the classical DGF definition (in terms of dialysis requirement) is also 1 week, so in our view f-DGF is a more functional definition which uses the same time frame.

Parameters characterizing the individual kidney during perfusion – such as vascular resistance, and flow and perfusate viability markers – were not used as potential predictors of outcome. In a separate analysis, renal resistances during MP were shown to correlate with DGF and 1 year graft survival (univariate analysis), but not with PNF.[150] Hence, further analysis of these parameters and perfusate biomarkers might help to identify kidneys at risk for DGF and PNF, also recently shown in an experimental study.[151-153] Interestingly, but not fully analyzed yet, was that kidneys with DGF after MP and after CS were seldom from the same donor in this study. This could imply that parameters of MP providing a prognosis for the development of DGF in the perfused kidney will in most cases not help to identify renal grafts at risk for DGF after CS.

A striking fact is that the proportion of ECDs in Germany, where donation is only allowed after brain death, is almost 50% (47.7% in 2006 and 48.2% in 2007) (Eurotransplant analysis). The proportion of ECDs in the main study was 27.9% (94/336) when donation after cardiocirculatory death was included and 30.9% (91/294) when only donation after brain death was considered. This relatively small proportion of ECDs is an effect caused by the procurement policy in Belgium and the Netherlands and could imply a strong bias toward better-quality ECD categorized donors in the present study. It can be assumed that ECD populations in other countries are not fully comparable to our study's inclusions and, therefore, the effect of MP versus CS as described in this article could be somewhat different.

The high rate of exclusion could represent a possible bias, but is explained by the early randomization process and high exclusion rate because one or both kidneys were eventually not transplanted. Exclusion for a combined organ transplantation was rare. This too could provide a bias toward the 'better' expanded criteria donor, and perhaps effects of MP could be even more pronounced in a series with more marginal ECD kidneys.

There were no kidney pairs that could not be randomized. All consecutive ECD donor kidney pairs were assessed for inclusion, randomized if they met the initial inclusion criteria, and only if vascular anatomy of the kidney randomized for MP prevented a reliable connection of the kidney to the perfusion machine, could the preservation methods for this pair be switched, thus indeed frustrating randomization. We checked whether the presence of vascular anomalies had any relevant influence on posttransplant outcome, and this was not the case. Therefore, the few cases in which preservation methods were switched did not introduce any bias to our results.

The recent review of Yuan *et al*. critically describes the possibilities and developments in the field of MP over the last decades. The authors emphasize the importance of investigating the relevance of MP for marginal donor organs. We feel that the present study adds important new data which support the benefit of MP for the preservation of such donor organs.[154]

In summary, this study shows that MP reduces the risk of DGF and improves 1-year graft survival and function in ECD kidneys. The development of better pretransplant predictors for DGF[155] could increase the cost-effectiveness of MP in expanded criteria donation. We believe that as long as there are no such reliable predictors, every ECD kidney should be machine perfused, because in the first year after transplantation alone, 12% more grafts could be saved as a result of MP.

Chapter 8

Machine perfusion or cold storage in deceased-donor kidney transplantation 3-year follow-up

Submitted for publication

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In 2009, we showed in an international randomized controlled trial that hypothermic machine perfusion of deceased-donor kidneys significantly reduced the risk of delayed graft function compared to cold storage preservation. We also found that 1-year graft survival was significantly better after machine perfusion.[48] As preservation related effects have so far been shown to affect early function only, we decided to extend the follow-up period of our study and investigate whether this substantial graft survival advantage would persist 3 years after transplantation.

In our study, one kidney of each included donor was randomly assigned to machine perfusion, and the contralateral organ to cold storage. For the present analysis, all 60 collaborating transplant centers were contacted. Three-year follow-up data were collected of all 672 recipients of consecutive kidneys donated after brain death or after cardiocirculatory death in the main data set, as well as 164 recipients of kidneys donated after cardiocirculatory death in the extended data set. End points were 3-year graft survival, patient survival, and serum creatinine. Statistical analyses were performed using the same methodology as described in our previous paper.[48]

Overall, 3-year graft survival was better for machine perfused kidneys (91% vs. 87%, adjusted hazard ratio for graft failure 0.60, p=0.04). Differentiated to donor type, 3-year graft survival after machine perfusion was superior to that after cold storage for kidneys donated after brain death (91% vs. 86%, adjusted hazard ratio 0.54, p=0.02), but not for kidneys donated after cardiocirculatory death. The 3-year graft survival advantage after machine perfusion was most pronounced for kidneys recovered from expanded criteria donors,[12] (86% vs. 76%, adjusted hazard ratio 0.38, p=0.01) (Figure 1a-d). Delayed graft function had a profound impact on graft survival of kidneys donated after brain death (Figure 1e). Three-year patient survival and serum creatinine were equal in the two study arms.

We conclude that, 3 years posttransplant, graft survival of kidneys donated after brain death remained significantly better after machine perfusion compared to cold storage, especially in kidneys recovered from expanded criteria donors. Delayed graft function was associated with a notably lower graft survival of kidneys donated after brain death. Despite the large reduction in delayed graft function by machine perfusion in kidneys donated after cardiocirculatory death that we showed earlier,[97] we found no beneficial effect of machine perfusion on graft survival in this subgroup. This could suggest a different type of delayed graft function in kidneys donated after cardiocirculatory death versus those donated after brain death.

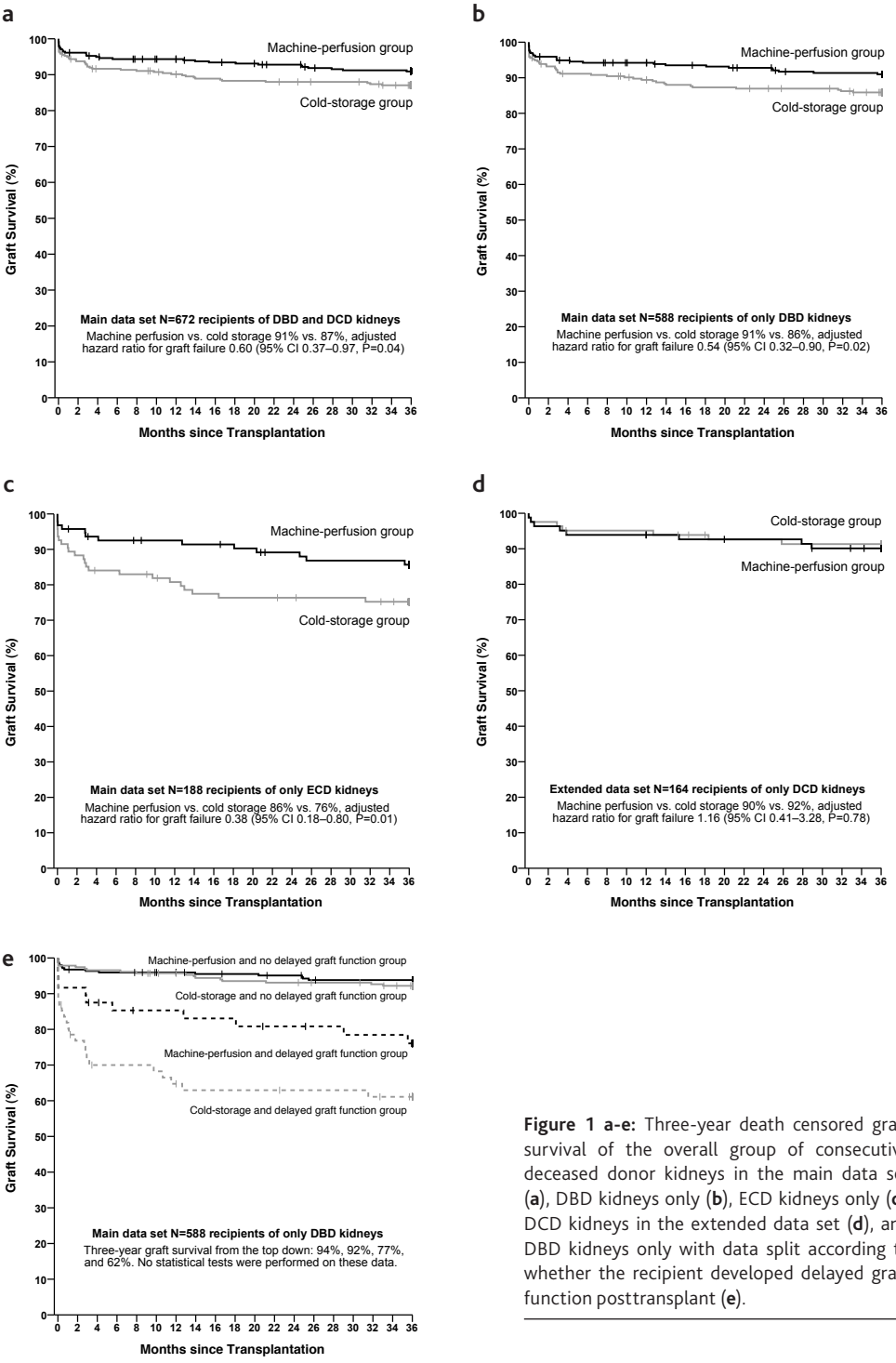

Figure 1 a-e: Three-year death censored graft survival of the overall group of consecutive deceased donor kidneys in the main data set (**a**), DBD kidneys only (**b**), ECD kidneys only (**c**), DCD kidneys in the extended data set (**d**), and DBD kidneys only with data split according to whether the recipient developed delayed graft function posttransplant (**e**).

Chapter 9

Cost-effectiveness of hypothermic machine preservation versus static cold storage in renal transplantation

Submitted for publication

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ABSTRACT

Static cold storage (CS) is the most widely used organ preservation method for deceased donor kidney grafts but there is increasing evidence that hypothermic machine perfusion (MP) may result in better outcome after transplantation. We performed an economic evaluation of MP versus CS alongside a multi-center RCT investigating short and long term cost-effectiveness. 336 consecutive kidney pairs were included, one of which was assigned to MP and one to CS. The economic evaluation combined the short term results based on the empirical data from the study with a Markov model with a 10-year time horizon. Direct medical costs of hospital stay, dialysis treatment and complications were included. Data regarding long-term survival, quality of life, and long term costs were derived from literature. The short-term evaluation showed that MP reduced the risk of delayed graft function and graft failure at lower costs than CS. The Markov model revealed cost savings of € 86,750 per life-year gained in favor of MP. The corresponding incremental cost-utility ratio was minus € 496,223 per quality-adjusted life-year gained. We conclude that life-years and QALYs can be gained while reducing costs at the same time, when kidneys are preserved by MP instead of CS.

INTRODUCTION

Preservation of the kidney graft while it is transferred from donor to recipient is a critical and vulnerable phase. Traditionally, static cold storage (CS) is the method of choice, involving cooling with one of several cold preservation solutions available, and transportation on melting ice. Modifications of this procedure, e.g. by changing the type of preservation solution, have been shown to have an impact on costs and effectiveness of transplantation.[103,104] Over the past decade, retrospective evidence has been accumulating suggesting that hypothermic machine perfusion (MP) of the kidney may result in better short term outcome than CS, with lower rates of delayed graft function (DGF) irrespective of whether kidneys are recovered from donors after cessation of circulation and cardiac death (DCD) or after brain death (DBD). MP involves the continuous pumping and recirculation of a preservation solution through the vasculature of the organ at temperatures between 1 and 10 °C, using a mechanical perfusion device.[54] As a result of promising initial reports, interest in MP has been rising worldwide, especially since the average age of deceased kidney donors has been increasing and the inherently elevated exposure to more concomitant morbidity has been associated with an additional detrimental effect on graft quality and function.[40,52,147]

Despite this preponderance of favorable data, a systematic review by Wight *et al*. concluded that insufficient high-quality prospective trials were available to allow firm conclusions about the clinical benefits of MP over CS, particularly with respect to delayed graft function and graft survival.[106] In addition, existing economic evaluations were considered to be of poor quality, i.e. not based on randomized studies.

Recently, our group has added evidence on the beneficial clinical effects of MP over CS with a large randomized controlled trial (RCT),[48] that showed that MP results in a reduced risk of DGF and an improved graft survival versus CS in the first year after transplantation for all deceased donor kidneys, irrespective of the donor type (DBD or DCD). To consolidate the evidence and underpin policy decisions regarding reimbursement of MP we have now performed an economic evaluation of MP versus CS using the data of this clinical trial, and expanded our analysis with a Markov model to estimate long-term cost-effectiveness and cost-utility of MP versus CS in kidney transplantation.

METHODS

The economic evaluation was based on the data from the recent international RCT.[48] In short, kidney pairs from consecutive deceased donors aged 16 years or older from the participating regions in the Netherlands, Belgium and the federal state of North Rhine-Westphalia in Germany were transplanted into two different recipients in the Eurotransplant region (Austria, Belgium, Germany, Luxemburg, the Netherlands, and Slovenia) after randomization to CS for one kidney and to MP for the contralateral kidney of each pair. Types of transplantation

included kidneys recovered from donors after cessation of circulation and cardiac death (DCD) or after brain death (DBD) and extended-criteria kidneys (ECD, donor age of 60 years or more or a donor age between 50 and 60 years, with at least two of the following additional donor characteristics: history of hypertension, death due to a cerebrovascular cause, and a serum creatinine level of more than 132 µmol per liter (1.5 mg per deciliter) before removal of the kidney).

Hypothermic pulsatile machine perfusion with the modified University of Wisconsin preservation solution was performed using LifePort Kidney Transporter machines (Organ Recovery Systems, One Pierce Place, Itasca IL, USA). Cold storage was performed according to established Eurotransplant protocols. The primary endpoint of the study was delayed graft function, defined as the requirement for dialysis during the first week after transplantation. Follow-up data until 1 year after transplantation were collected from the participating centers in 100% of the cases through a secure online database.

Economic evaluation

The economic evaluation was performed in a dual approach. Short-term cost-effectiveness, i.e. the costs and effects up to 1 year after transplantation, was evaluated based on data from the clinical study, using the percentage of functioning grafts as the primary outcome. Data regarding graft function (delayed graft function, primary non-function) and dialysis treatment during this period were used to calculate the costs per patient. Data regarding graft survival and costs were used to calculate the incremental cost-effectiveness ratio (ICER) by dividing the difference in total costs over 1 year ($Costs_{MP} - Costs_{CS}$) by the difference in functioning grafts ($Grafts_{MP} - Grafts_{CS}$) after 1 year. Direct - non-incremental - costs related to kidney transplant surgery and immunosuppressive drugs were not included in the economic evaluation.

For the evaluation of the long-term effects a Markov simulation model was constructed.[156] It encompassed the definition of three discrete states of patients, i.e. functioning graft, graft failure and death. The model had a cycle length of 1 year and transition probabilities derived from literature and/or clinical expertise were used to determine the annual flow of patients between states.

Short-term evaluation

Costs were calculated from a hospital perspective: direct medical costs associated with hospital stay, dialysis treatment[157] and complications were included. The price level of 2007 was used. Prices from previous publications were transformed to the year 2007 by indexing according to the Dutch consumer price index.

Since a few participating centers did record return to dialysis after primary or late graft failure, but failed to record all subsequent dialysis treatments, some follow-up data on dialysis were missing. In view of the importance of these data for the cost-effectiveness estimates these missing values were replaced by estimates of the expected number of dialysis

treatments based on established clinical practice. For each week of missing data concerning dialysis treatments, three hemodialysis treatments were added, and costs were calculated accordingly. In case of primary non-function (PNF) and graft failure after a period of function, missing values were replaced until 1 year posttransplant or until death of the patient. For hospital readmission days, missing values were replaced by zero based on the assumption that any readmission would have been registered and missing database entries would only occur if readmission had not taken place.

Peritoneal dialysis per day		€ 109
Hemodialysis per treatment		€ 465
Renogram		€ 218
Renal angiography		€ 326
Renal ultrasound		€ 62
Renal biopsy		€ 280
Graft removal (nephrectomy)		€ 1,464
Hospital admission per day		€ 505
Preservation - machine perfusion		
LifePort annual depreciation	€ 2,880	
Tx per year (2006, NL)	360	
Number of machines (NL)	12	
Costs per Tx		€ 96
Disposables, including fluids		€ 635
Transport costs		€ 111
Total costs MP preservation per Tx		€ **842**
Preservation - cold storage		
Preservation fluid		€ 147
Disposables		€ 20
Total costs CS preservation per Tx		€ € **167**

Table 1: Unit costs and prices in the short-term evaluation.
Tx denotes transplantation

Unit costs used to calculate the treatment costs are presented in Table 1. Immediate costs of graft failure were calculated in accordance with a standard protocol established by consensus between participating centers and countries (renogram, renal ultrasound and renal biopsy), and graft removal in case of definitive non-function. Separate calculations were made for Germany and Belgium based on data provided by the centers in those countries (see supplemental information).

For the calculation of the costs of organ preservation we used a preliminary estimate of the purchase price of the LifePort preservation machine (€ 14,400). Annual depreciation

was assumed to be 20% in accordance with general guidelines for technical equipment. The costs per transplant further depended on the number of transplants per year and the number of machines required to perfuse all kidneys involved in these transplants in the Dutch trial region. Costs of transportation of the empty preservation machine back to the hospital of origin were also calculated for the Dutch situation (80% of transplantations within the country and 20% outside in other countries of the Eurotransplant region, average travel distance 800 kilometers). The standalone LifePort device requires no additional perfusionist or transport logistics than for CS transport, so personnel and transportation costs from donor to transplant center were not included.

For static CS, preservation costs were calculated based on the costs of preservation fluids and the costs of disposables used for packaging the organ. Histidine-tryptophan-ketoglutarate (HTK) was considered to be used for 50% of the kidneys (€ 69.95 per liter) and University of Wisconsin (UW) solution in the other 50% of kidneys (€ 224.20 per liter). Costs of disposables for CS were estimated at € 20.00 per kidney.

Bootstrap analysis

To evaluate uncertainty surrounding the ICER, a bootstrap analysis was performed.[158,159] This method implies replication of datasets from the original study data, simulating repetition of the study. Although this method gives an indication of the expected range of the ICER, it cannot be used to reduce uncertainty regarding the clinical outcome, e.g. in case of low numbers of events such as in our DCD subgroup. Therefore bootstrapping was not performed in this subgroup. For the overall results, as well as for the subgroup of patients receiving a kidney retrieved from extended criteria donation we performed bootstrapping with 5,000 replications and plotted the results in a cost-effectiveness plane.

Long-term evaluation

The annual transition probabilities, costs and utilities are presented in Table 2 (see Appendix for further details). For each year that a simulated patient was in a certain state, costs corresponding to this state were calculated. In addition, transition costs were calculated for transition from functional to failure (i.e. costs associated with diagnosis and implications of failure) and for the transition to death.

The total costs per year were summed up to present total costs of transplantation for the CS arm and the MP arm of the clinical study's cohort of kidney recipients. Summation was done with and without discounting, i.e. with and without reflecting depreciated value of future costs and effects. The net effect of discounting is that early costs and effects receive more weight in the summation than late costs and effects. Common annual discount rates in economic evaluations are 3 to 5%. A similar depreciation of future health outcomes, i.e. life-years and QALYs, was applied.

The model was populated with patients in various states according to the 1-year data from the RCT. In the cold storage arm there were 296 functional grafts, 31 failures and 9

deaths; in the machine perfusion arm there were 309 functional grafts, 16 failures and 11 deaths. The average patient age at the start of the Markov model simulation was set at 50 years. A background age-specific mortality rate for 5-year categories was included in the model (source: CBS (Statistics Netherlands), www.cbs.nl). In view of the assumed age of entry into the model and the limited availability of long-term estimates of patient and graft survival, the time horizon of the long-term evaluation was restricted to 10 years posttransplant.

Transition probabilities	**Value**	**Source**
Functional to graft failure		
- Cold storage arm	0.076	baseline risk 5% annually (UNOS), tripled risk for patients after DGF (26.5% of patients)[48]
- Machine preservation arm	0.071	baseline risk 5% annually (UNOS), tripled risk in 20.8% of patients
	0.066	multivariate estimate[48]
General		
- graft failure to death		
- first transplant	0.15	Liem *et al.*[161]
- re-transplant	variable	average of background and Liem *et al.*
- graft failure to re-transplant	0.15	UNOS transplant and waiting list data
- re-transplant to graft failure	1.85	relative to initial failure risk[48]
State/transition costs	**Value**	**Source**
- functional	€ 10,324	annual costs after transplantation[157,162]
- transition to failure	€ 2,024	immediate failure costs (short-term analysis)
- failure (dialysis)	€ 83,599	annual costs dialysis[157]
- re-transplant	€ 51,619	De Wit *et al.*[157]
- death	€ 1,000	--
State utilities	**Value**	**Source**
- QALY in functional state	0.90	De Wit *et al.*[157]
- QALY after graft failure	0.66	general population value, CHD[157]
- QALY after re-transplant	0.90	return to functional level assumed
- QALY death	0	--

Table 2: Transition probabilities, costs and utilities for the long term model.

Statistical analysis

The data were analyzed at the level of the recipient, regarding the recipient couples with a kidney from the same donor as independent. For the short-term analysis, cost data were calculated based on individual health care consumption data using the SPSS statistical package, version 16.0. The bootstrap analyses were performed using R-project software, version 2.5.1.

	ECD		DCD	
	MP (n = 94)	CS (n = 94)	MP (n = 42)	CS (n = 42)
DGF	22 (23.4%)	29 (30.9%)	22 (52.4%)	28 (66.7%)
PNF	3 (3.2%)	11 (11.7%)	1 (2.4%)	1 (2.4%)
Graft failure < 1 yr	8 (8.5%)	18 (19.1%)	2 (4.8%)	3 (7.1%)
Dialysis costs	€ 3,581	€ 9,986	€ 3,992	€ 5,369
Early dysfunction	€ 178	€ 344	€ 328	€ 408
Readmission costs	€ 2,561	€ 2,792	€ 1,466	€ 1,514
Preservation costs	€ 842	€ 167	€ 842	€ 167
Total costs	€ 7,162	€ 13,289	€ 6,628	€ 7,458

Table 3: Average effects and costs per patient for expanded criteria donation (ECD) and donation after cardiocirculatory death (DCD).

RESULTS

Short-term evaluation

The data from the clinical study showed that MP significantly reduced the risk of DGF (20.8% versus 26.5% univariate estimate, P = 0.046; multivariate estimate adjusted OR 0.57, P = 0.01) and more than halved the incidence of primary non-function after transplantation (2.1% versus 4.8%, P = 0.08), when compared to CS. Furthermore, MP significantly reduced the duration of DGF (median 10 versus 13 days, P = 0.04). One-year allograft survival was significantly better in the machine perfusion group (94% versus 90%, P = 0.04). In a multivariate Cox model, MP also significantly reduced the risk of graft failure up to 1 year posttransplant compared to CS (adjusted hazard ratio 0.52, P = 0.03).

The mean costs in the CS arm were € 8,053 versus € 6,180 in the MP arm (see Appendix for details). The main components of total costs were costs of dialysis (€ 5,405 for CS versus € 3,130 for MP, after imputation of missing data) and readmission costs (€ 2,263 for CS versus € 2,062 for MP). These cost differences were used for the bootstrap analyses together with actual graft survival after 1 year (Figure 1, panel a). Each dot represents the ICER of a replicated bootstrap sample. The white dot in the middle represents the estimated average ICER (-50,251 Euro/additional functioning graft, non-parametric 95% confidence interval -151,382 – 35,558). Clearly, the majority of the replications (93.9%) results in lower costs for MP, and an even larger proportion (97.0%) shows better graft survival for MP. The combination of better graft survival and lower costs occurs in 92.9 % of replications.

A separate bootstrap analysis for ECD transplants is presented in panel b. The underlying cost and effectiveness data, including those for DCD transplants, are presented in Table 3. For

ECD, the results were even more favorable than the overall results, both with respect to the differences in effects and the difference in costs.

Outcome	Conservative	Adjusted
Incremental costs		
undiscounted	€ -3,381,907	€ -4,447,707
discounted*	€ -3,020,963	€ -3,865,575
Incremental life-years		
undiscounted	49.76	59.61
discounted*	37.34	44.56
Incremental QALYs		
undiscounted	11.90	12.88
discounted*	7.29	7.79

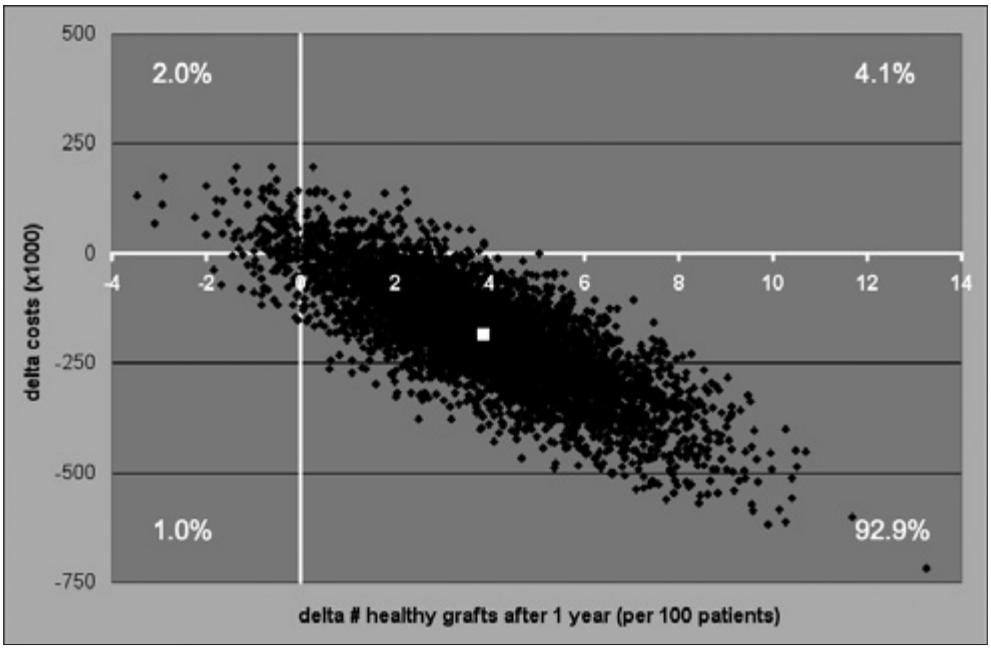
Table 4: Markov model estimates of incremental costs, life-years and QALYs for crude and adjusted effect size estimates# with a time horizon of 10 years.
Crude and adjusted (multivariate) estimation of DGF risks with MP (see Table 2)
* Discount rate: 4%

Long-term evaluation

The main results of the long-term model are summarized in Table 4. They reveal a clear cost difference in favor of MP as well as positive incremental life-years and QALYs, indicating gains in patient survival and QALYs for MP compared to CS, resulting in a cost-effectiveness ratio of minus € 86,750 per life-year gained and a cost-utility ratio of minus € 496,223 per quality-adjusted life-year gained.

Sensitivity analyses

Sensitivity analyses were performed with variations of various parameters for the short-term evaluation (e.g. national cost differences, costs of equipment and materials) and the long-term evaluation (crude versus corrected effect estimates, time horizons of 5 and 8 years, variable discount rates, see Appendix). Only changes to the costs of machine perfusion disposables (organ cassette plus preservation solution) had a profound impact on the cost-effectiveness. Bootstrapping showed that the likelihood of cost-effectiveness decreased to 67% if the price of disposables was doubled and to 47% if the price was tripled (see Appendix).



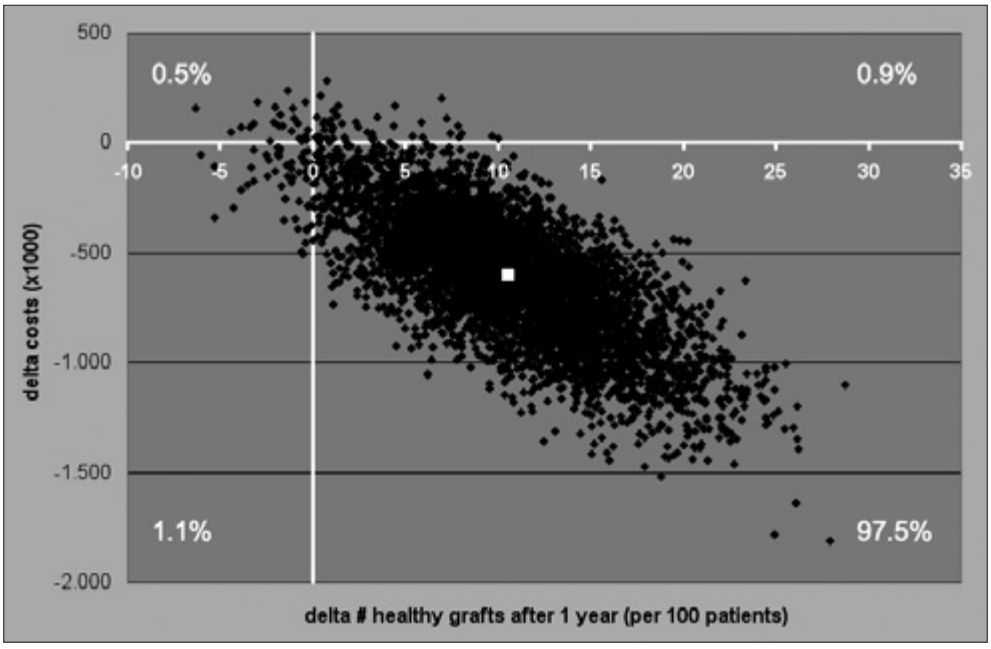


Figure 1: Results of bootstrap analysis of the short-term cost-effectiveness of MP versus CS. Percentages indicate proportion of simulations in the respective quadrants. **(a)** Overall results after imputation. **(b)** Patients receiving a kidney retrieved from expanded criteria donation (ECD).

DISCUSSION

This is the first economic evaluation of MP versus CS based on an international prospective RCT. Both the short-term and the long-term analyses show that MP dominates CS, i.e. results in lower costs and better outcomes in the mixed overall population of deceased donor kidneys irrespective of donor type (DBD or DCD) and for both standard and extended donor criteria. For the short-term analysis, the advantage is expressed as a higher proportion of functioning grafts after 1 year with MP versus CS, while average costs over the first year were lower for MP than for CS. This applies especially for the subgroup of ECD transplants (n=188). For DCD transplants (n=84) the low number of graft failures (n=5) prevents firm conclusions. For the long-term analysis, the advantage of MP is expressed as better survival (life-years) both with and without correction for quality of life. Although largely insensitive to changes in discount rates, time horizon, and national cost and clinical policy differences, the outcome was very sensitive to increases in costs of MP disposables.

The short-term results are derived directly from the data in the RCT by Moers *et al*.[48] with respect to graft function and several cost-related variables such as dialysis requirement and duration of hospitalization. The data collection for some non-endpoint variables (such as the number of dialysis treatments after graft failure) was not entirely complete, and as a result assumptions had to be made in order to allow analysis of all cases. This shortcoming of the data collection process is significant, since return to dialysis constitutes the major part of costs associated with graft failure. However, the cost-effectiveness of MP was also superior to CS without imputation of these data. Imputation of missing values associated with hospital readmission by replacing them with zero obviously lowered the mean costs of readmission in both groups, but the difference between the groups remained around € 200. Therefore, this correction is not expected to affect the overall results.

Exploration of differences in cost-effectiveness for donor type (ECD and DCD) showed remarkable results. For ECD transplants, the dominance of MP over CS was even more convincing than in the total population. For DCD, the benefit of MP in terms of costs and effects was minimal. This finding may be explained by the fact that DGF is usually associated with only a limited number of dialysis treatments posttransplant (until sufficient graft function occurs), whereas a graft failure implies dialysis for a much longer period (i.e. until end of follow-up, retransplant, or death). Although in the DCD subgroup MP was associated with a 17-fold reduced risk of DGF,[48] no benefit of MP in terms of graft survival was found. As a result, cost differences caused by dialysis treatments remained relatively low between the two study arms. A recent study by Watson *et al*. with a sample size comparable to our study (45 pairs of DCD kidneys) showed considerably worse effectiveness of MP in DCD donation with respect to DGF than our study.[160] These differences may be related to differences in ischemia and other disturbances of the kidneys in both studies. It can be expected that more frequent occurrence of DGF would further deteriorate the cost-effectiveness of MP in DCD.

The long term model required more assumptions and relied more on data from the literature than the short-term evaluation. Extrapolation to longer time horizons showed that the advantage of MP over CS continued to exist, but this observation should be interpreted with caution since reliable long-term survival data are very scarce. In a recent HTA report by Bond *et al*. with an economic model which took into account some of the preliminary data from our study, the importance of reliable long-term survival data was also stressed.[47] Contrary to what was estimated by Wight *et al*. in their review performed in 2003,[106] our data indicate that MP may be cost-effective in the short-term, and not only in the long term.

Overall, the results of our analyses suggest that implementation of MP preservation of all common types of deceased donor kidneys is likely to be cost-effective with lower costs per life-year and reduced costs per QALY compared to CS preservation. Although this analysis was focused on the Dutch situation, explorative analyses showed that adaptation to local costs and procedures for Germany and Belgium had no major impact on the results. Analysis of further scenarios with different numbers of MP equipment in relation to number of annual transplants suggest that the economic superiority of MP over CS is unlikely to be affected. As our sensitivity analyses demonstrate, only substantial increases in costs of disposables could affect the short-term cost-effectiveness of machine perfusion over static cold storage.

SUPPLEMENTARY APPENDIX

Mean costs per treatment arm

The mean costs per treatment arm are tabulated below. The effect of imputation of missing data for dialysis treatment was considerable. Of the 23 subjects with PNF, 15 had missing data regarding dialysis. These caused average imputed costs of € 792 in the MP arm and € 1,839 in the CS arm. Of those patients with graft failure other than PNF, 16 had missing data, leading to costs of approximately € 100 for MP and € 1,500 for CS. After addition of actual and imputed costs of dialysis, there was a difference of about € 1,800 in favor of MP. As a result, addition of the costs of preservation and graft failure management (ultrasound, biopsy, etcetera) resulted in an average cost difference of nearly € 1,600 per transplant in favor of MP.

	MP-arm		CS-arm	
Dialysis (uncorrected)	n = 334	€ 746	n = 331	€ 980
Imputed (PNF, n=15)	n = 336	€ 1,009	n = 336	€ 1,839
Imputed (later failure, n=16)	n = 336	€ 1,023	n = 336	€ 1,549
Dialysis (actual+imputed)	n = 336	€ 2,773	n = 336	€ 4,354
Preservation	n = 336	€ 842	n = 336	€ 167
Early dysfunction (DGF/PNF)	n = 336	€ 147	n = 336	€ 218
Subtotal		€ 3,762		€ 4,739
Readmission (imputed)	n = 336	€ 2,062	n = 336	€ 2,263
Total costs (imputed)		**€ 5,824**		**€ 7,002**

Average costs per patient by treatment

Due to the fact that data on hospital readmission were missing in a number of cases, the overall total costs could only be reliably calculated if readmission costs were imputed. For the sake of clarity, readmission costs with and without imputation are shown.

International variations in costs

For Belgium, this consisted of one additional ultrasound and biopsy, and weekly biopsies in case of persistent DGF. For Germany, the number of additional biopsies was 1.5 (the average of the specified number of 1-2 per patient), and one weekly biopsy plus two weekly ultrasounds in case of persistent DGF. In addition, 12.5% of German patients (1 in 8) were estimated to undergo a renal angiography. Fixed additional costs of biopsy and ultrasound were added to immediate failure costs. Variable costs of weekly procedures depending on length of DGF were added for a maximum period of 4 weeks. If graft survival was less than 4 weeks, variable costs were reduced accordingly. The results of these additions are presented as part of the sensitivity analyses.

Transition probabilities, utilities and costs for the long-term model

The base-case transition probabilities from functional to failure were based on the crude univariate risk of DGF in both groups: 20.8% in the MP arm and 26.5% in the CS arm. This corresponds to a relative risk of 78.5% and an odds ratio (OR) of 0.728. Therefore, this is a conservative estimate compared to the OR reported for the multivariate comparison, which was 0.57.

Patient survival rates were based on data by Liem *et al*.[161] for the first transplant and a variable survival rate was used after re-transplant. An annual probability of re-transplantation was included based on expert opinions and the probability of graft failure after re-transplantation was estimated to be 1.85 times the risk after the first transplant, as observed in the clinical study. Annual costs after transplantation were derived from De Wit *et al*.[157] and Hilbrands *et al*.[162] Costs of dialysis were calculated in an identical fashion as for the short-term model. Transition costs consisted of costs of graft failure, as in the short-term evaluation, and the costs of death, for which there were no good sources available. Since sensitivity analyses indicated that the results were largely insensitive to changes in these costs (less than 0.5% of total costs), an arbitrary amount of € 1,000 was used.

Utilities were based on quality of life measurements in patients with end-stage renal disease performed by De Wit *et al*.[157] using the following instruments: EuroQol (EQ-5D) Instrument,[163,164] Standard Gamble,[165] and Time Trade Off.[166] The EQ-5D is a widely accepted 5-item generic questionnaire to measure quality of life. The last two methods are preference based measurements, allowing the expression of quality of life as a single indicator – usually a number between 0 and 1 – with 0 representing death and 1 representing full health. This single indicator can be used for the calculation of (cost per) QALY. In addition to the end-stage renal disease patients, De Wit *et al*. also used data from a UK population sample on the valuation of health states[167,168] and applied these to the health status as described by the patients. The quality of life experienced by these patients was used for the state of failure after transplantation in the present study. Utilities for a successful transplant were also derived from De Wit *et al*, who assumed that quality of life after transplantation was close to that in the general population based on previous literature.[169-171]

Sensitivity analyses

Apart from the bootstrap analysis of the short-term outcome and the effect of various effect sizes and time horizons in the long-term analysis presented above, the impact of country-specific variations of procedures in case of graft failure was calculated. For Belgium, the costs of failure-related procedures were € 324 higher in the MP arm and € 400 higher in het CS arm. For Germany, costs were slightly higher (€ 356 and € 434, respectively). The total costs increased by about 6%, and the ICERs for Belgium and Germany were -3,137,500 and -3,170,000 Euro per additional patient with a functional graft at 1 year, respectively. The change compared to the ICER without country-specific additional costs was 6.5% and 7.6%, respectively.

The impact of assumptions made in the long term model was evaluated in a number of ways. First, the influence of the effect of MP versus CS was explored by using both the crude (univariate) and the adjusted (multivariate) risk of graft failure, constituting a 2-fold increase of the difference in failure rate. The impact of these two different estimates on the cost-effectiveness and cost-utility ratios was minimal.

Second, the impact of variable discount rates and time horizons was explored. The results of these analyses showed that the number of life-years gained increased when the time horizon was expanded, reflecting a persistent advantage of MP over CS with respect to patient survival in the long term.

The impact of variations of the costs of equipment and materials for preservation was also assessed. If the ratio of machines to transplants was changed from 1 to 30 to 1 to 15, the costs per transplant increased by € 96. The same effect occurred if the purchase price of the LifePort machine was doubled. Changes of this magnitude are very small in relation to the total costs calculated. Changes to the costs of machine perfusion disposables (organ cassette plus preservation solution) will have a more profound impact since they will be entirely added to the costs per individual kidney. This is illustrated in the table below, which shows the consequences of doubling and tripling the price of these disposables on average costs and on the probability of MP remaining cost-effective.

	MP-arm		CS-arm	
Dialysis (actual+imputed)	n = 336	€ 2,773	n = 336	€ 4,354
Early dysfunction (DGF/PNF)	n = 336	€ 147	n = 336	€ 218
Readmission (imputed)	n = 336	€ 2,062	n = 336	€ 2,263
Subtotal per patient		€ 4,982		€ 6,835
Actual current costs of disposables: € 635				
Preservation		€ 842		€ 167
Total costs per patient		€ 5,824		€ 7,002
Likelihood of cost-effectiveness (bootstrap): 86%				
Doubled costs of disposables: € 1,270				
Preservation		€ 1,477		€ 167
Total costs per patient		€ 6,459		€ 7,002
Likelihood of cost-effectiveness (bootstrap): 67%				
Tripled costs of disposables: € 1,905				
Preservation		€ 2,112		€ 167
Total costs per patient		€ 7,094		€ 7,002
Likelihood of cost-effectiveness (bootstrap): 47%				

Sensitivity analysis of various price levels of MP disposables

Chapter 10

The value of machine perfusion perfusate biomarkers for predicting kidney transplant outcome

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ABSTRACT

Background

Retrospective evidence suggests that lactate dehydrogenase (LDH), aspartate amino-transferase (ASAT), total glutathione-S-transferase (GST), alanine-aminopeptidase (Ala-AP), *N*-acetyl-β-D-glucosaminidase (NAG), and heart-type fatty acid binding protein (H-FABP) measured during kidney machine perfusion could have predictive value for posttransplant outcome. However, these data may be biased due to organ discard based on biomarker measurements, and previous analyses were not adjusted for likely confounding factors. No reliable prospective evidence has been available so far. Nevertheless, some centers already utilize these biomarkers to aid decisions on accepting or discarding a donor kidney.

Methods

From 306 deceased donor kidneys donated after brain death or controlled cardiac death and included in an international randomized controlled trial, these six biomarkers were measured in the machine perfusion perfusate. In this unselected prospective data set, we tested whether concentrations were associated with delayed graft function, primary non-function, and graft survival. Multivariate regression models investigated whether the biomarkers remained independent predictors when adjusted for relevant confounding factors.

Results

GST, NAG, and H-FABP were independent predictors of delayed graft function, but not of primary non-function and graft survival. LDH, ASAT, and Ala-AP had no independent prognostic potential for any of the end points. Perfusate biomarker concentrations had no relevant correlation with cold ischemic time or renal vascular resistance on the pump.

Conclusions

Elevated GST, NAG, or H-FABP concentrations during machine perfusion are an indication to adjust posttransplant recipient management. However, this study shows for the first time that perfusate biomarker measurements should not lead to kidney discard.

INTRODUCTION

Recently, we conducted an international randomized controlled trial (RCT) investigating the effect of hypothermic machine perfusion (MP) versus static cold storage in deceased donor kidney transplantation. We found that MP reduced the risk of delayed graft function (DGF) with an odds ratio (OR) of 0.57 for all common donor types equally, and duration of DGF was three days shorter in MP kidney recipients. In addition, graft survival after MP was significantly better already at 1 year posttransplant, and MP reduced the risk of graft failure with a hazard ratio of 0.52.[48] Together with evidence coming from earlier studies,[46,105] these findings may lead to an increased usage of MP.

In addition to the beneficial effect that MP preservation has on postoperative outcome, many centers have advocated the method as a diagnostic tool to evaluate graft quality before transplantation. Several groups have suggested that perfusion characteristics, such as intrarenal vascular resistance, could have a predictive value for posttransplant outcome.[106,122] In addition, evidence points out that perfusate biomarkers during MP may have a prognostic potential,[172-174] and as a result some centers use such measurements to aid decisions on transplanting or discarding a kidney. Nevertheless, the published data are scarce, using only retrospective data, and suffer from selection bias. Moreover, statistical analyses have been univariate, so far. Hence, likely confounding factors may have had an effect on the reported association between perfusate biomarkers and posttransplant results. No previous studies have addressed the question whether such measurements have a truly independent prognostic relevance.

In the present study, we have analyzed data from the MP-arm of our RCT to investigate whether six important perfusate biomarkers that have been advocated and are already in use by various centers have any true independent predictive value for renal transplant outcome. We deliberately chose not to search for novel biomarkers, but to test established biomarkers for the first time with multivariate analyses in a unique and unselected prospective data set.
We have studied six biomarkers that are commonly associated with renal cellular injury in general, and tubular damage in particular. The extent of such injury is thought to be a major cause of DGF and graft failure.[3,31,175] Lactate dehydrogenase (LDH) is a non-specific cellular injury marker, but since perfusate samples were collected from an isolated kidney perfused on the pump, LDH release could reflect general renal injury. Aspartate aminotransferase (ASAT) is an enzyme that facilitates the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate. Although clinically most often associated with the liver, ASAT is also found in renal parenchymal cells. ASAT is associated with acute damage to parenchymal cells.[176] Glutathione-S-transferase (GST) is an enzyme localized in the renal tubules. It is involved in deconjugation of waste products and excreted into the urine.[177] Although α-GST is most directly associated with proximal tubular injury, total GST (the sum of α-GST and π-GST) is easier to measure. Total GST has been shown to also reliably reflect renal tubular injury and has become the most often used biochemical marker for kidney injury assessment during MP.

In the present paper, the abbreviation GST refers to total GST. Alanine-aminopeptidase (Ala-AP) is an exopeptidase with a role in cell regulation and is also excreted into the urine.[178] Ala-AP release is associated with renal tubular injury. *N*-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme present in various tissues in the body, including the kidney, and its release is also associated with ischemic tubular damage.[179] Heart-type fatty acid binding protein (H-FABP) is a cytosolic protein, located in the distal renal tubules and involved in free fatty acid transport from the cytosol to mitochondria, and is mainly found in the urine.[180] Elevated H-FABP release has been associated with ischemic kidney tissue injury.[181]

METHODS

Donors and recipients

As previously published,[48] a total of 376 deceased donor kidney pairs were included in the extended dataset of our RCT between November 1, 2005 and August 17, 2007. Of these inclusions, 294 were donors after brain death (DBD), and 82 were controlled DCD (Maastricht category III). One graft of each donor's kidney pair was cold stored, and the contralateral organ was preserved by MP. For the present study, we analyzed perfusate biomarkers and follow-up data of the recipients in the MP-arm of our trial. For detailed information on study design, randomization, logistics, and data collection, we refer to our previous publication.[48]

Machine perfusion

LifePort Kidney Transporter® machines (Organ Recovery Systems, Itasca, IL, USA) were used for perfusion, delivering a pulsatile flow of University of Wisconsin MP solution (Kidney Preservation Solution-1®)[108] at 1–8°C, with a systolic perfusion pressure fixed at 30 mmHg. Kidneys were machine perfused immediately following organ retrieval and flush-out, until transplantation. To prevent bias in clinical decisions about transplanting or discarding an organ, intravascular resistance and flow readings, as well as biomarker concentrations were never revealed to the transplantation team.

Sample collection and biochemical analysis

Perfusate samples of 10 ml were drawn after 10 minutes, after 1 hour, and at the end of the preservation period just prior to transplantation. All samples were stored on ice during transport, and thereafter at -80°C until further analysis. Details on the methodology of biochemical analysis are provided in the supplementary appendix.

	Overall (N = 306)	DBD (N = 231)	DCD (N = 75)	IF (N = 230)	DGF (N = 76)	PNF (N = 7)
Donor demographics						
Donor age[a] (yr)	50 (16–78)	52 (16–78)	43 (17–65)	50 (16–77)	50 (18–78)	44 (37–63)
Female donor (%)	39	42	28	39	40	57
DCD donor (%)	25	0	100	15	53	29
ECD donor[b] (%)	28	32	16	29	25	43
Traumatic cause of death (%)	23	22	27	24	21	0
Donor history of hypertension (%)	22	26	12	24	18	57
Donor history of diabetes mellitus (%)	5	5	5	4	9	0
Recipient demographics						
Recipient age[a] (yr)	53 (11–79)	54 (11–79)	51 (24–73)	53 (11–79)	54 (13–73)	46 (13–67)
Female recipient (%)	42	43	37	44	36	71
Total time spent on the waiting list[a] (yr)	5 (1–8)	5 (1–8)	5 (2–8)	5 (1–8)	5 (1–8)	7 (3–8)
Previous transplants (% ≥1)	31	24	56	25	53	43
PRA level >5% (%)	13	13	13	13	20	43
Immunosuppressive drugs (%)						
Prednisolone	98	97	99	97	99	100
Cyclosporine	47	47	48	46	53	43
Tacrolimus	51	51	49	51	49	57
Azathioprine	1	<1	1	<1	1	0
Mycophenolate mofetil	88	85	93	87	88	100
Antithymocyte globulin	14	13	15	14	15	29
Transplant demographics						
HLA mismatches (% of 0 mismatches)	13	17	3	13	13	14
Cold ischemic time[a] (h)	15 (4–30)	15 (4–30)	16 (4–27)	15 (4–30)	16 (8–27)	16 (14–25)
Posttransplant outcome						
Delayed graft function (%)	25	16	53	0	100	100
Duration of delayed graft function (days)[a]	10 (1–93)	9 (3–31)	10 (1–93)	n/a	10 (1–93)	n/a
Primary non-function (%)	2.3	2.2	2.7	0	9.2	100
Any acute rejection in first year (%)	23	24	21	19	36	14
1 year death censored graft survival (%)	95	95	93	97	87	0

Table 1: Donor, recipient, and transplant demographics and overall posttransplant outcome for the study group (n = 306 transplants in the MP-arm of the prospective study). Figures are presented as those for the whole group (Overall), as well as separate characteristics for kidneys derived from donation after brain death (DBD) and donation after cardiac death (DCD), and for patients with immediate function (IF), delayed graft function (DGF), or primary non-function (PNF). No statistical tests were performed on the data in this table. Note that the PNF group consists of only 7 cases, which makes a comparison with other groups less reliable. n/a denotes not applicable.

[a] Median (range).

[b] ECD denotes expanded criteria donation, which was defined as donor age ≥60, or donor age between 50 and 60, with at least two of the following additional donor characteristics: (1) history of hypertension, (2) cerebrovascular cause of death, (3) pre-retrieval serum creatinine >132 µmol/l.

Study end points

Delayed graft function (DGF) and primary non-function (PNF) were analyzed as outcome measures of short-term graft function. DGF was defined as dialysis requirement in the first week posttransplant. PNF was scored when a kidney graft never showed sufficient function to prevent the need for dialysis after transplantation. Death censored graft survival (GS) served as end point for graft performance up to 1 year posttransplant.

Statistical analysis

First, univariate comparisons were made for each biomarker. The Mann-Whitney test investigated whether biomarker concentrations were significantly different in recipients with and without DGF and PNF. We used the Kaplan-Meier method with logrank tests to obtain a univariate comparison of graft survival up to 1 year between recipients of kidneys with a biomarker value below and above the median.

Second, for each individual biomarker, logistic regression models were constructed to find independent risk factors for DGF, and Cox proportional hazards models were used to identify independent risk factors for graft failure.[77] Apart from the biomarker of interest, other covariates in these models were: Renal vascular resistance at the end of MP (mmHg/ml/min), donor age (yr), donor type (DCD vs. DBD), CIT (hr), the duration of pre-transplant dialysis (yr), the number of previous transplants of the recipient, recipient age (yr), and the number of HLA mismatches. These particular covariates were chosen for the models of the present study because they were significant predictors of early posttransplant outcome in our data set.[48] To prevent overfitting of the models, other covariates that had no significant impact on outcome in the RCT were not considered in the multivariate analyses of the present study. For all multivariate analyses, except those for NAG, biomarker concentrations had to be log-transformed to better approach a normal distribution.

Two-sided p-values under 0.05 were considered to indicate statistical significance.

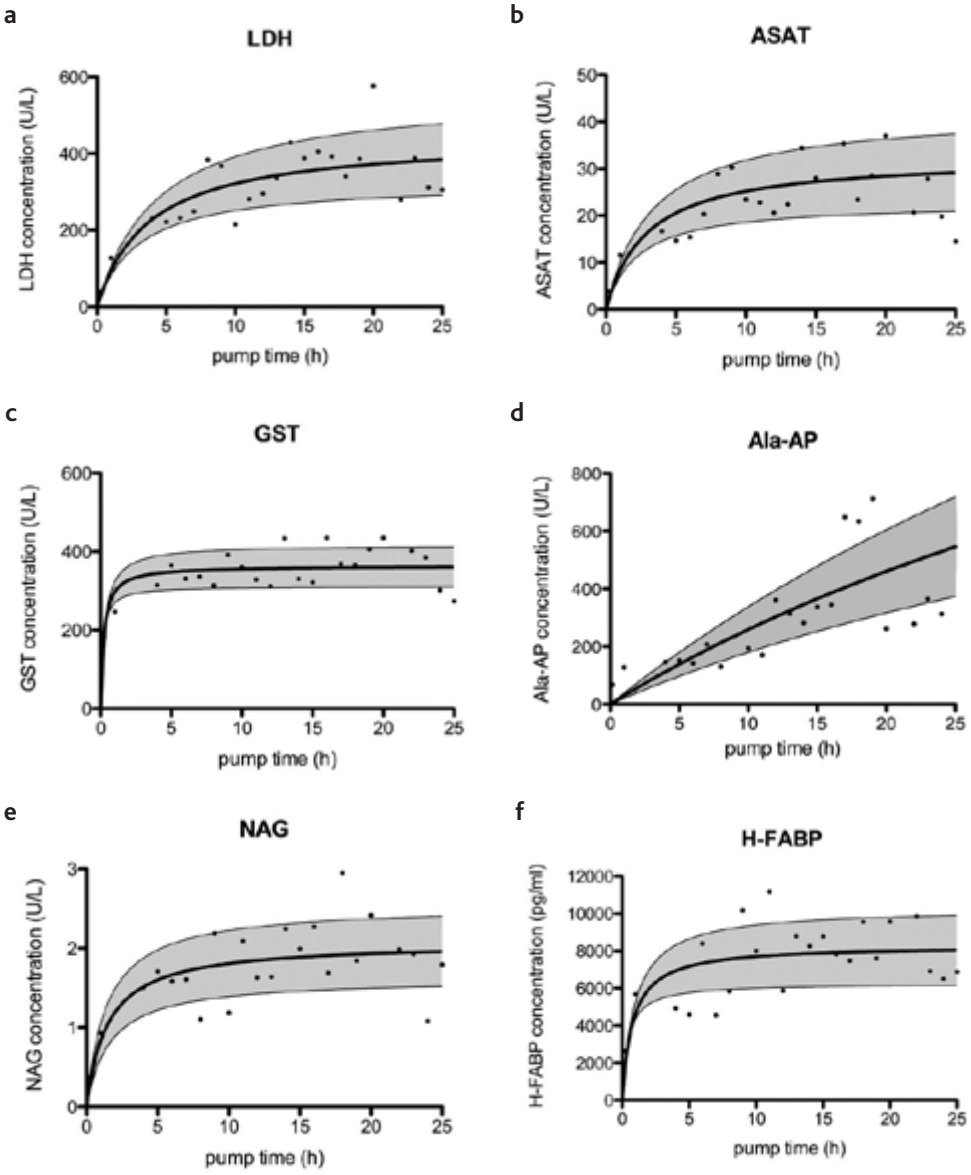

Figure 1: Evolution of each biomarker's perfusate concentration in time. Bullets represent mean biomarker concentrations per time point after the initiation of MP. Bold lines are a least square fit to the plotted data points, with thin upper and lower lines and a gray area indicating plus and minus standard error of the mean. The baseline function used for each least square fit was a typical equation for molecular saturation in fluids: $y = ax / (x + b)$, where a and b are determined by the least square method. Curves were corrected for outliers using Dixon's Q test.

RESULTS

In 306 out of 376 kidney transplants in the MP-arm of the RCT suitable perfusate samples were available for biomarker analysis. Table 1 shows baseline characteristics and outcome of these transplants. The baseline values did not differ significantly from the characteristics of the whole MP-arm of 376 cases.

We found that the concentration of most biomarkers, except for Ala-AP, did not change considerably after four to six hours of MP (Fig. 1). This finding is further supported by the observation that there was no relevant correlation between cold ischemic time (CIT) and the concentration of any of the six perfusate biomarkers measured at the end of MP.

Table 2

Biomarker concentration after 1 h of MP:	**Overall**		
Lactate dehydrogenase (U/l)	95 (57–151)		
Aspartate aminotransferase (U/l)	8 (5–13)		
Glutathione-S-transferase (U/l)	218 (172–280)		
Alanine-aminopeptidase (U/l)	80 (43–143)		
N-acetyl-β-D-glucosaminidase (U/l)	0.70 (0.46–1.14)		
Heart-type fatty acid binding protein (pg/ml)	4,340 (2,794–5,950)		
Biomarker concentration after 1 h of MP:	**no DGF**	**DGF**	**P-value**
Lactate dehydrogenase (U/l)	91 (55–146)	104 (71–167)	0.089
Aspartate aminotransferase (U/l)	8 (5–12)	8 (6–14)	0.070
Glutathione-S-transferase (U/l)	214 (169–278)	235 (202–297)	0.026
Alanine-aminopeptidase (U/l)	81 (38–147)	75 (45–131)	0.61
N-acetyl-β-D-glucosaminidase (U/l)	0.70 (0.45–1.14)	0.70 (0.47–1.26)	0.98
Heart-type fatty acid binding protein (pg/ml)	4,018 (2,692–5,832)	4,914 (3,422–6,244)	0.028
Biomarker concentration after 1 h of MP:	**no PNF**	**PNF**	**P-value**
Lactate dehydrogenase (U/l)	95 (57–148)	145 (44–175)	0.54
Aspartate aminotransferase (U/l)	8 (5–13)	8 (5–13)	0.98
Glutathione-S-transferase (U/l)	218 (172–281)	227 (164–307)	1.00
Alanine-aminopeptidase (U/l)	80 (42–145)	49 (25–97)	0.27
N-acetyl-β-D-glucosaminidase (U/l)	0.70 (0.46–1.14)	0.49 (0.44–1.42)	0.72
Heart-type fatty acid binding protein (pg/ml)	4,339 (2,804–5,966)	4,885 (2,115–5,656)	0.82
Biomarker concentration at end of MP:	**Overall**		
Lactate dehydrogenase (U/l)	304 (185–456)		
Aspartate aminotransferase (U/l)	19 (12–33)		
Glutathione-S-transferase (U/l)	324 (261–398)		
Alanine-aminopeptidase (U/l)	246 (137–423)		
N-acetyl-β-D-glucosaminidase (U/l)	1.44 (0.93–2.49)		
Heart-type fatty acid binding protein (pg/ml)	5,851 (4,442–8,608)		

Table 2 Continued

Biomarker concentration at end of MP:	no DGF	DGF	P-value
Lactate dehydrogenase (U/l)	285 (173–415)	358 (227–529)	0.015
Aspartate aminotransferase (U/l)	18 (12–28)	25 (14–43)	0.006
Glutathione-S-transferase (U/l)	302 (256–382)	379 (308–465)	<0.0005
Alanine-aminopeptidase (U/l)	241 (122–420)	253 (184–444)	0.10
N-acetyl-β-D-glucosaminidase (U/l)	1.33 (0.91–2.22)	1.98 (1.21–3.39)	0.001
Heart-type fatty acid binding protein (pg/ml)	5,178 (4,120–7,980)	7,325 (5,020–12,248)	<0.0005
Biomarker concentration at end of MP:	**no PNF**	**PNF**	**P-value**
Lactate dehydrogenase (U/l)	304 (182–456)	276 (213–675)	0.88
Aspartate aminotransferase (U/l)	19 (12–33)	21 (8–26)	0.71
Glutathione-S-transferase (U/l)	324 (261–401)	291 (213–368)	0.39
Alanine-aminopeptidase (U/l)	243 (133–422)	313 (182–470)	0.25
N-acetyl-β-D-glucosaminidase (U/l)	1.43 (0.93–2.44)	2.50 (1.23–3.76)	0.11
Heart-type fatty acid binding protein (pg/ml)	5,855 (4,442–8,582)	5,833 (4,315–10,253)	0.80

Table 2: Univariate characteristics of biomarkers after 1 h and at the end of MP. Values are expressed as median (interquartile range).

Univariate tests

Table 2 shows that kidneys that developed DGF after transplantation were those that had significantly higher GST and H-FABP concentrations already after 1 h of MP. Since these two biomarkers appeared to show such an early discriminative potential, we also tested whether their concentrations in donor plasma just prior to organ retrieval were higher for kidneys that developed DGF after transplantation versus kidneys with immediate function. No significant difference was detected (also see the supplementary appendix). At the end of MP, all biomarkers except Ala-AP had a significantly higher median perfusate concentration for kidneys that developed DGF versus grafts with immediate function. In contrast, at both time points, there was no difference in biomarker release between kidneys that did and did not develop PNF. The Kaplan-Meier analyses (figures provided in the supplementary appendix) showed that death censored graft survival up to 1 year after transplantation was not significantly different for grafts with any biomarker concentration (end of MP) above the median, versus those with concentrations below the median. Receiver-operator curves (ROC) investigating each biomarker's predictive accuracy for DGF yielded areas-under-the-curve of 0.60 for LDH, 0.61 for ASAT, 0.67 for GST, 0.57 for Ala-AP, 0.64 for NAG, and 0.64 for H-FABP (Fig. 2). In the Supplemental Digital Content we show Pearson's correlation coefficients between the six biomarkers measured and for each biomarker's correlation with renal vascular resistance at the end of MP and with CIT. None of the biomarkers had a relevant correlation with renal resistance or with CIT. The strongest correlation that we found was the one between LDH and ASAT (0.56). Supplemental figures show that (except for Ala-

AP) the curve of each biomarker's evolution over time was significantly higher for kidneys that developed DGF versus those with immediate function and for DCD versus DBD kidneys.

	Odds ratio / Hazard ratio	
Biomarker covariate	**(95% CI)[b]**	**P-value**
Risk of delayed graft function		
(biomarker measured after 1 h of MP)		
Lactate dehydrogenase (log[U/l])	1.43 (0.94–2.19)	0.10
Aspartate aminotransferase (log[U/l])	1.34 (0.90–2.00)	0.16
Glutathione-S-transferase (log[U/l])	1.90 (0.82–4.42)	0.14
Alanine-aminopeptidase (log[U/l])	0.81 (0.57–1.17)	0.26
N-acetyl-β-D-glucosaminidase (U/l)	1.17 (0.78–1.76)	0.45
Heart-type fatty acid binding protein (log[pg/ml])	1.27 (0.84–1.93)	0.26
Risk of delayed graft function		
(biomarker measured at end of MP)		
Lactate dehydrogenase (log[U/l])	1.09 (0.68–1.74)	0.73
Aspartate aminotransferase (log[U/l])	0.97 (0.63–1.51)	0.91
Glutathione-S-transferase (log[U/l])	3.21 (1.37–7.50)	0.007
Alanine-aminopeptidase (log[U/l])	1.03 (0.70–1.49)	0.90
N-acetyl-β-D-glucosaminidase (U/l)	1.31 (1.04–1.66)	0.02
Heart-type fatty acid binding protein (log[pg/ml])	1.91 (1.18–3.08)	0.008
Risk of graft failure within the first year posttransplant[c]		
(biomarker measured at end of MP)		
Lactate dehydrogenase (log[U/l])	0.94 (0.43–1.97)	0.83
Aspartate aminotransferase (log[U/l])	0.74 (0.36–1.50)	0.40
Glutathione-S-transferase (log[U/l])	0.31 (0.06–1.49)	0.14
Alanine-aminopeptidase (log[U/l])	1.05 (0.55–2.02)	0.89
N-acetyl-β-D-glucosaminidase (U/l)	1.06 (0.86–1.32)	0.57
Heart-type fatty acid binding protein (log[pg/ml])	0.81 (0.41–1.60)	0.54

Table 3: Multivariate risk analysis[a] for delayed graft function and graft failure. For each of the six biomarkers, a separate multivariate model was built. In this table only the adjusted odds / hazard ratios and p-values for the biomarker of interest are shown.

[a] Logistic regression models for delayed graft function, and Cox proportional hazards models for graft failure. Other covariates in each model were: Renal vascular resistance at the end of MP (mmHg/ml/min), donor age (yr), donor type (DCD vs. DBD), CIT (hr), the duration of pre-transplant dialysis (yr), the number of previous transplants of the recipient, recipient age (yr), and the number of HLA mismatches.

[b] Odds ratios apply to the logistic regression model and hazard ratios apply to the Cox proportional hazards model.

[c] Censored upon death with a functioning graft.

Multivariate models

The logistic regression and Cox proportional hazards models (table 3) showed that only GST, NAG, and H-FABP levels in the perfusate measured at the end of MP were true independent predictors for the risk of DGF. None of the six biomarkers had any significant independent predictive value for the risk of graft failure in the first year posttransplant.

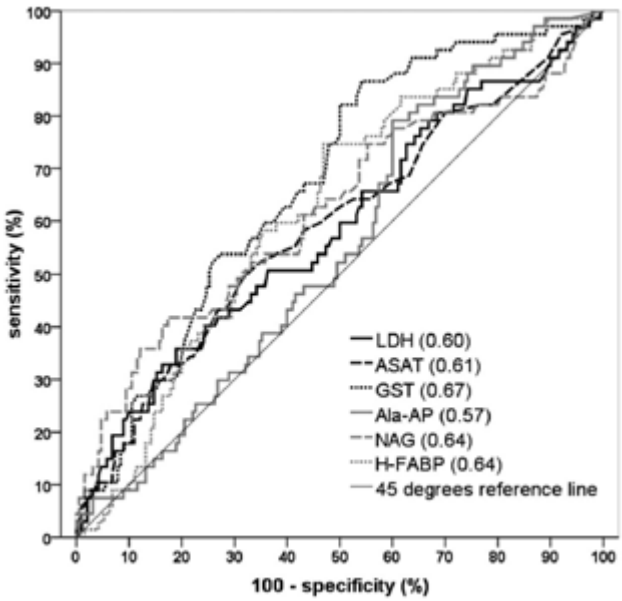

Figure 2: Receiver-operator curves for each of the six perfusate biomarkers' concentration at the end of MP at a continuous range of cut-off points. The numbers between brackets indicate the area-under-the-curve for each line.

DISCUSSION

Predicting outcome after kidney transplantation has been the topic of numerous, often retrospective, studies. Well-known pertinent donor and recipient factors, as well as cold and warm ischemic time and the organ preservation modality are usually included into such multivariate risk assessments. Recently, Rao *et al*. developed a comprehensive risk score to predict renal graft failure,[182] and Moore *et al*. conducted a study comparing the predictive value of several existing risk scores for early graft (dys)function.[183] Both studies yielded useful tools to aid decisions on organ acceptance and early recipient management. However, current risk scores are no more than a sophisticated mathematic compilation of routinely collected variables that are already known to the transplant team. In an attempt to add something genuinely novel to the decision-making process, several groups have introduced various biomarkers that may have predictive potential for short and long term outcome. For example, evidence suggests that donor serum interleukins could be indicative for posttransplant complications.[184] In the past, Daemen *et al*. and Gok *et al*. have performed analyses of the biomarkers GST, H-FABP, Ala-AP, and/or LDH in renal MP perfusate. Their studies found that biomarker concentrations were elevated in perfusates of those kidneys that were discarded on other grounds, and that uncontrolled (Maastricht cat. II) DCD grafts tended to have higher GST, H-FABP, and Ala-AP concentrations in the perfusate than kidneys recovered from controlled (cat. III) DCD procedures. In addition, LDH and GST levels correlated with warm ischemic time.[172-174,184,185] However, these studies did not investigate whether any of these biomarkers was *independently* associated with outcome after transplantation. It is very plausible that an association between the concentration of a certain substance in the perfusate and posttransplant results is no more than a surrogate marker for another underlying causal factor already known. For example, a longer warm ischemic time could result in an increased biomarker release into the MP perfusate due to more ischemic injury to the kidney. In that case, measuring these markers will not provide any extra information to the clinician, since simply considering warm ischemic time would be sufficient to appreciate the amount of injury to the graft. With this in mind, the univariate results of the present study could be biased by confounding factors such as DCD versus DBD: As our supplemental figures show, DCD kidneys release significantly more injury biomarkers into the perfusate, but such kidneys are already known to have inferior posttransplant outcome in terms of more DGF. Measuring perfusate biomarkers is only worth the extra effort and expense when the biomarker of choice has a truly independent predictive value in the context of traditional prognostic factors. Therefore, multivariate analyses that correct for such likely confounding factors are essential to appreciate any biomarker's true prognostic potential.

This is the first prospective study which shows that GST, NAG, and H-FABP, measured in the MP perfusate at the end of MP, are independently associated with the risk of DGF, and that LDH, ASAT, and Ala-AP do not seem to have such predictive potential. Therefore, measuring the former three markers will indeed provide an extra piece of information to clinicians who

care for a kidney recipient. Nevertheless, since no marker could predict graft survival, we feel that there is no rationale to discard a kidney based on such measurements. DGF may be an unwelcome postoperative complication, but given the present donor organ shortage a known elevated risk of DGF will seldom be the reason to refuse a renal graft. Several centers worldwide already use one of the perfusate biomarkers discussed in this paper for pretransplant kidney quality assessment to aid decisions on acceptance or discard of donor kidneys. The results of the present analysis are of immediate clinical importance, since our data suggest that this so called "evidence based" decision making is probably not justified. Prior knowledge of an increased DGF risk could, however, be useful to fine-tune recipient management. Our data set is likely to be more reliable and has no selection bias compared to other studies: Data collection for the present study was multicenter, prospective, and no kidneys were discarded based on biomarker measurements or other MP related characteristics.

With ROC analysis we sought for relevant cut-off values for each biomarker's concentration.[186] In the present analysis, all areas-under-the-ROC were well under 0.8, with the value for GST (0.67) most approaching a reliable predictive test. These results do not allow to determine cut-off values for the three predictive biomarkers, since either sensitivity or specificity will be poor. A more practical approach could be to consider the biomarker's concentration as a continuous variable in the context of other predictive factors. As usual, it is the clinician's task to make a balanced judgement of DGF risk, taking all such relevant factors into account.

The univariate results of the present study suggest that GST and H-FABP have significantly higher levels in the perfusate already after 1 h of MP in kidneys that develop DGF. However, our multivariate assessment shows that this association does not persist when tested against relevant confounding factors. In addition, GST or H-HABP measured in donor plasma did not predict DGF. Therefore, measuring these biomarkers in the donor or already after 1 h of MP may be too early to draw reliable conclusions about DGF risk.

Interestingly, no biomarkers correlated with renal intravascular resistance during MP. This finding suggests that perfusate biomarkers reflect a different aspect of renal injury than intravascular resistance does. Perfusate markers in our study are most likely related to tubular injury, whereas vascular resistance may reflect endothelial damage.

A possible limitation of this study is that the most important time point at which three biomarkers were independent predictors of DGF (i.e. end of MP) was not standardized, and could be anywhere between four and 25 hours after initiation of MP. This may have introduced more variance in these data. However, none of the six markers had a relevant correlation with CIT, and we found that five out of six curves showing the average accumulation of each of the biomarkers' concentration in time followed an almost horizontal course after four to six hours of MP. This is in line with previous findings.[185] Therefore, any time point after four to six hours is likely to be suitable to take a representative perfusate biomarker sample. This will add to clinical applicability, as organ transport logistics will not always allow perfusate sampling at a fixed time point. Since an elevated perfusate GST, NAG, or H-FABP concentration will at best

lead to adjusted recipient management and not to kidney discard or re-allocation, the rather late availability of test results at the end of MP should not be a major concern for clinicians. It is important to note that the incidence of PNF was very low in our data set. Hence, comparisons between groups are unreliable for this end point. Nevertheless, since there was a considerable number of graft failures in the first year posttransplant, 1-year graft survival does provide a reliable end point in our data to assess this single most important outcome measure after transplantation.

Another limitation of our data is that this study did not include uncontrolled (Maastricht cat. I and II) DCD kidneys. These are the organs that have sustained most ischemic damage, rendering viability testing during MP even more relevant.[40,185] In addition, it is conceivable that a biomarker will be predictive for graft failure in the context of such extremely marginal kidneys. Similar multivariate analyses with cohorts of recipients of these organs are needed to determine whether pefusate markers could predict more than just DGF. Unfortunately, to date, large series of such transplantations remain rare.

In conclusion, the present study demonstrated that GST, NAG, and H-FABP released in the perfusate and measured at the end of machine perfusion are independent predictors for DGF, but not for graft survival in the first year after kidney transplantation. Since their prognostic value for DGF is at best moderate, these markers should always be considered in the context of other known variables. LDH, ASAT, and Ala-AP do not posess independent predictive potential for posttransplant outcome. Given the results of our analysis, an elevated GST, NAG, or H-FABP concentration in the MP perfusate could be an additional trigger to adjust postoperative recipient management. However, in defiance of the practice in various transplant centers worldwide, this prospective study for the first time showed that the values of any of these markers may not be used for the decision to either transplant or discard a DBD or controlled DCD kidney.

SUPPLEMENTARY APPENDIX

Donor inclusions used for this study

We used the MP arm of the *extended* data set of our original study (376 recipients of a machine perfused kidney).[48] This extended set consists of the 336 DBD and DCD machine perfused kidney recipients who were included between November 1, 2005 and October 31, 2006 plus the machine perfused DCD kidney recipients who were included between November 1, 2006 and August 17, 2007 following an amendment to the study protocol. This amendment is described in the Results section of the NEJM paper (pages 10 and 13), as well as in that paper's figure 2, which shows subgroup analyses that were conducted on this extended data set of 752 recipients in total (i.e. 376 in the CS arm and 376 in the MP arm). All 376 recipients of machine perfused kidneys have therefore received a graft that was part of the same study, and those are exactly the kidneys that were also described in the NEJM paper. No additional inclusions were done solely for the biomarker analysis. For the present biomarker analysis, we chose to use this extended data set, as this would result in a superior statistical power to detect any effects, and there would be significantly more DCD kidney recipients to analyze (82 instead of 42). This higher percentage of DCD kidneys in the extended data set does also account for the somewhat higher overall DGF incidence in the extended data set versus the main data set of our RCT.

Biomarker concentration in donor plasma:	Overall
Glutathione-S-transferase (U/l)	252 (205–329)
Heart-type fatty acid binding protein (pg/ml)	2,272 (1,129–4,713)

Biomarker concentration in donor plasma:	no DGF	DGF	p-value
Glutathione-S-transferase (U/l)	261 (207–342)	242 (192–300)	0.081
Heart-type fatty acid binding protein (pg/ml)	2,111 (1,128–4,907)	2,450 (1,168–4,359)	0.97

Table S1: Univariate characteristics of GST and H-FABP measured in donor plasma just before organ retrieval. Values are expressed as median (interquartile range).

Biochemical analysis

LDH and ASAT were measured using standard automated clinical photometric kit assays on a Modular P800 analyzer (kit category nrs. 04794940/04796217 and 04571100/04571118, respectively, Roche Diagnostics, Mannheim, Germany). The activity of GST was measured spectrophotometrically (Power Wave 200 Spectrophotometer, Bio-Tek Instruments, Winooski, USA) at 340 nm via the conversion of 1-chloro-2,4-dinitrobenzene (CDNB) to glutathione-S-dinitrobenzene. The working substrate consisted of 0.1 ml of 100 mM CDNB added to 0.1 ml of 200 mM glutathione with 9.8 ml Dulbecco's phosphate buffered saline.

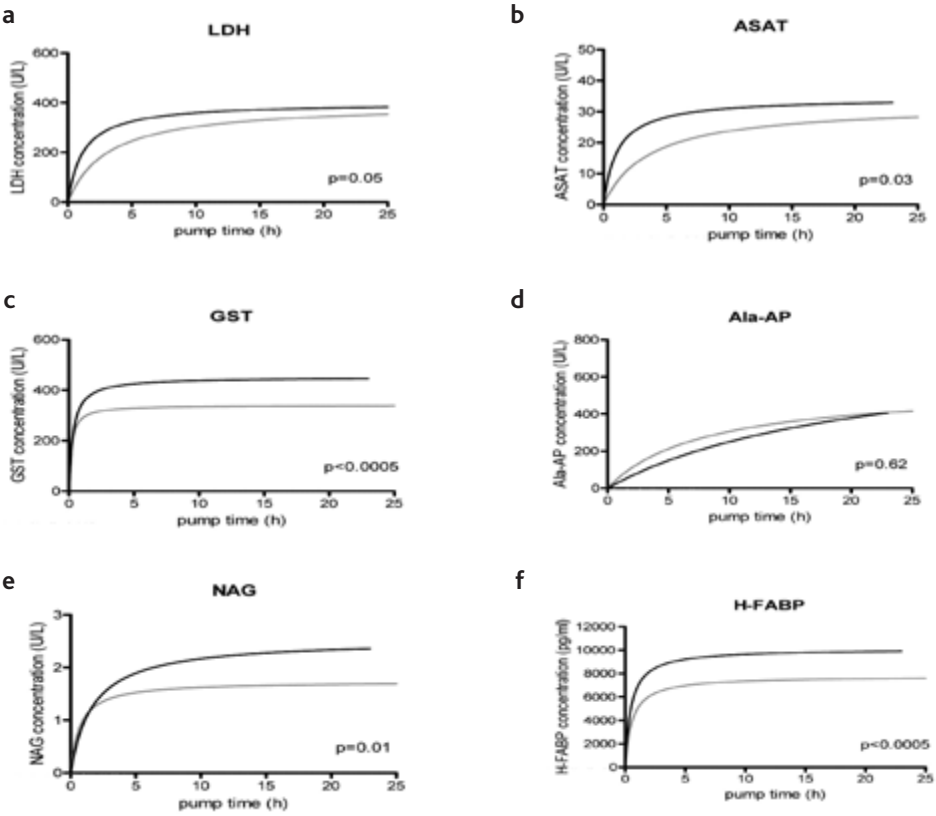

Figure S1: Evolution of each biomarker's perfusate concentration in time split into kidneys that developed DGF and kidneys with immediate function. Lines are a least square fit to mean biomarker concentrations per time point after the initiation of MP, with gray lines drawn for kidneys with immediate function and black lines for kidneys that developed DGF. The baseline function used for each least square fit was a typical equation for molecular saturation in fluids: $y = ax / (x + b)$, where a and b are determined by the least square method. Curves were corrected for outliers using Dixon's Q test. P-values are the result of Mann-Whitney tests which compared the areas-under-the-curve per group.

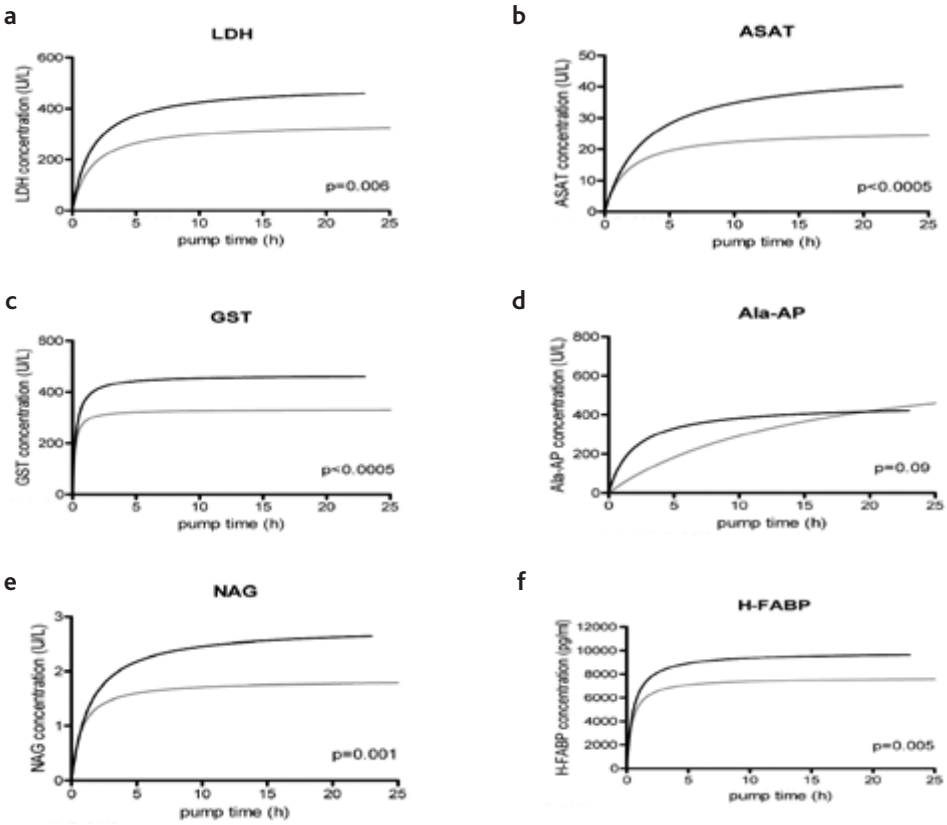

Figure S2: Evolution of each biomarker's perfusate concentration in time split into kidneys recovered from DBD and kidneys recovered from DCD. Lines are a least square fit to mean biomarker concentrations per time point after the initiation of MP, with gray lines drawn for DBD kidneys and black lines for DCD kidneys. The baseline function used for each least square fit was a typical equation for molecular saturation in fluids: $y = ax / (x + b)$, where a and b are determined by the least square method. Curves were corrected for outliers using Dixon's Q test. P-values are the result of Mann-Whitney tests which compared the areas-under-the-curve per group.

Biomarker covariate	Odds ratio (95% CI)	P-value
Risk of delayed graft function *(biomarker measured in donor plasma)*		
Glutathione-S-transferase (U/l)	1.00 (1.00–1.00)	0.22
Heart-type fatty acid binding protein (pg/ml)	1.00 (1.00–1.00)	0.36

Table S2: Multivariate risk analysis (logistic regression model) for delayed graft function testing whether GST or H-FABP concentrations in donor plasma just before organ retrieval had any independent prognostic value for the risk of developing DGF after transplantation. Other covariates in each model were: Renal vascular resistance at the end of MP (mmHg/ml/min), donor age (yr), donor type (DCD vs. DBD), CIT (hr), the duration of pre-transplant dialysis (yr), the number of previous transplants of the recipient, recipient age (yr), and the number of HLA mismatches. In this table only the adjusted odds ratios and p-values for the biomarker of interest are shown.

The final pH of the reaction was set at 6.5. Calibration was performed with GST prepared from human placenta tissue (Sigma Aldricht, Zwijndrecht, The Netherlands). H-FABP was measured using a commercially available human H-FABP enzyme-linked immuno sorbent assay (ELISA) kit (Hycult Biotechnology, Uden, The Netherlands). Activity of Ala-AP and NAG were measured using colorimetric assays.[187] NAG was measured using a modified enzyme assay according to Findlay[188] at pH 4.25 using *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide as a substrate. Ala-AP was detected with the modified enzymatic assay of Pfleiderer[189] using alanine-*p*-nitroanilide as a substrate.

	CIT	RR	H-FABP	NAG	Ala-AP	GST	ASAT
LDH	**0.15**	0.08	**0.39**	**0.24**	0.08	**0.45**	**0.56**
ASAT	0.08	0.10	**0.29**	**0.15**	0.09	**0.44**	
GST	0.06	0.05	**0.43**	**0.21**	**0.15**		
Ala-AP	0.11	-0.08	**0.16**	**0.28**			
NAG	0.11	0.08	**0.32**				
H-FABP	0.03	<0.01					

Table S3: Pearson's correlation coefficients between the six biomarkers at the end of machine perfusion, and for each biomarker's correlation with cold ischemic time (CIT) and renal vascular resistance (RR) at the end of machine perfusion. Numbers printed in bold indicate those correlations that were statistically significant ($p<0.05$).

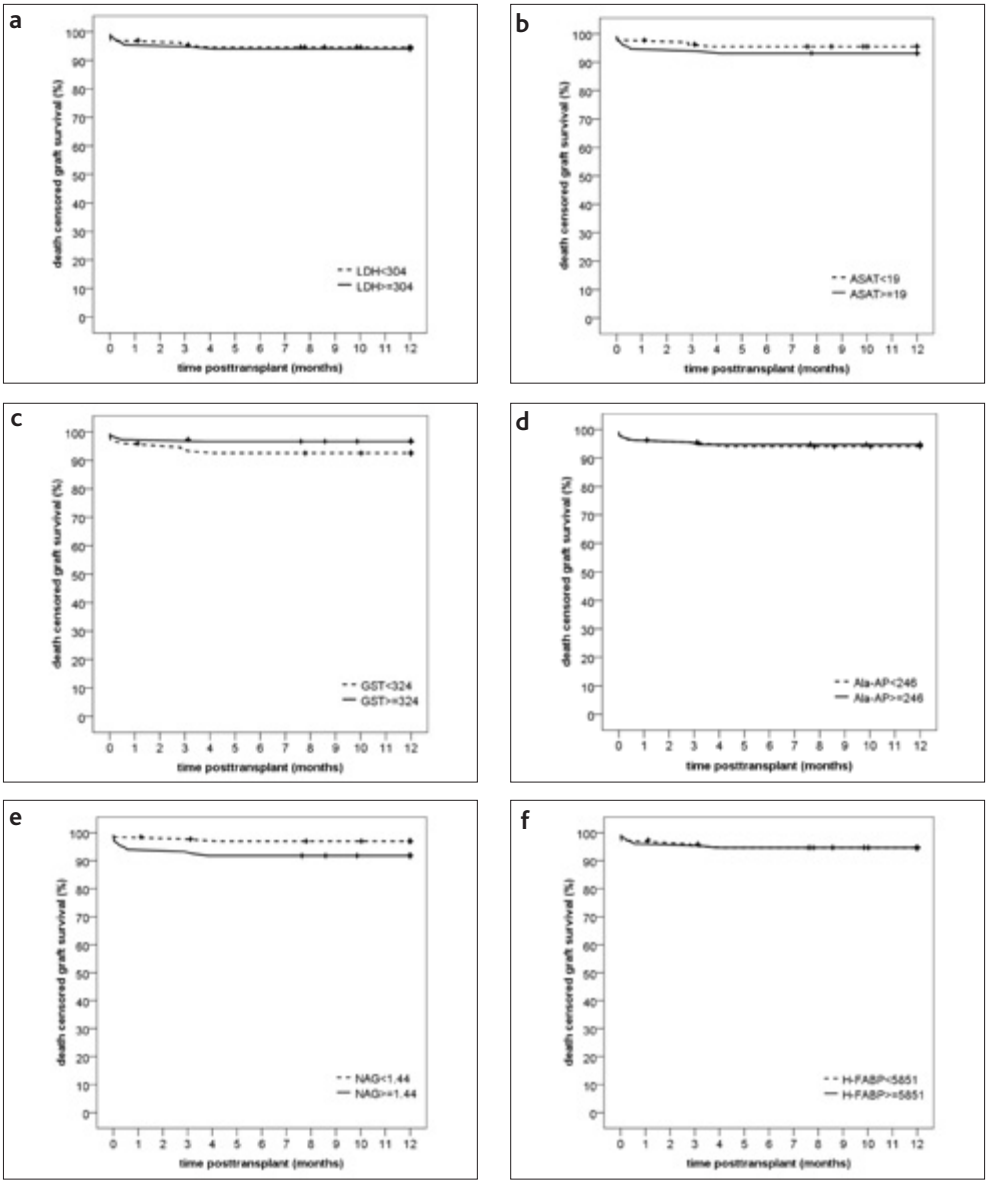

Figure S3: Kaplan-Meier plots of 1-year death censored graft survival split at each biomarker's median value. Logrank tests showed that all p-values for the difference between each pair of survival curves were above 0.05. Vertical lines indicate cases that were censored upon death with a functioning graft.

Chapter 11

The prognostic value of renal resistance during hypothermic machine perfusion of deceased donor kidneys

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ABSTRACT

Vascular renal resistance (RR) during hypothermic machine perfusion (MP) is frequently used in kidney graft quality assessment. However, the association between RR and outcome has never been prospectively validated. Prospectively collected RR values of 302 machine-perfused deceased donor kidneys of all types (standard and extended criteria donor kidneys and kidneys donated after cardiac death), transplanted without prior knowledge of these RR values, were studied. In this cohort, we determined the association between RR and delayed graft function (DGF) and 1-year graft survival. The RR (mmHg/mL/min) at the end of HMP was an independent risk factor for DGF (odds ratio 21.12 [1.03–435.0]; P = 0.048) but the predictive value of RR was low, reflected by a c-statistic of the receiver operator characteristic curve of 0.58. The RR was also found to be an independent risk factor for 1-year graft failure (hazard ratio 12.33 [1.11–136.85]; P = 0.004). Determinants of transplant outcome are multifactorial in nature and this study identifies RR as an additional parameter to take into account when evaluating graft quality and estimating the likelihood of successful outcome. However, RR as a stand-alone quality assessment tool cannot be used to predict outcome with sufficient precision.

INTRODUCTION

Hypothermic machine perfusion (MP) preserves kidney grafts by continuous or pulsatile administration of a recirculating cold (1–10°C) preservation solution. The Machine Preservation Trial (MP Trial) recently showed that MP decreases the incidence of primary non-function (PNF) and delayed graft function (DGF), and increases one-year graft survival compared to standard static cold storage.[48] In addition, MP offers the unique possibility to assess the graft in the interval between procurement and transplantation by monitoring perfusion dynamics and/or perfusate biomarkers that possibly correlate with graft outcome.

Since the early days of MP in the 1960s, it has been assumed that perfusion dynamics such as perfusion pressure, perfusate flow, and intravascular renal resistance (RR) can reliably predict kidney graft outcome. To a certain extent, there is indeed evidence that perfusion parameters correlate with kidney graft function. However, this evidence originates almost exclusively from retrospective studies in which kidneys were preselected and discarded based on empirically defined perfusion parameter thresholds.[105] Needless to say, systematically discarding kidneys introduced a major bias in these studies. Two of the earliest studies addressing the association between perfusion parameters and early graft function did not preselect kidneys based on these parameters. Henry *et al.* showed a correlation between early dysfunction and RR at the end of MP.[190] However, the group of kidneys with early dysfunction was small (n = 10) and only one of those kidneys developed PNF. Sampson *et al.* found no difference in flow rates between immediately functioning kidneys and those that developed early graft failure.[191] Noteworthy, 12 of the 18 graft failures in this study were related to hyperacute or acute rejection, and grafts were removed early after transplantation. The changed donor population and improved immunosuppression makes it even more difficult to interpret these results leaving the issue of the true prognostic value of perfusion parameters unresolved.

With the enduring donor shortage, kidneys of "uncertain" quality originating from expanded criteria donors (ECDs) or donated after cardiac death (DCD) are increasingly used. As these kidneys have a higher incidence of PNF and DGF compared with standard criteria donor (SCD) kidneys,[12,40] the need for specific and sensitive surrogates of their viability is becoming even more critical. The absence of prospective analyses of the association between perfusion parameters and kidney transplant outcomes, and the increasingly urgent need for valid predictors of graft outcome led us to analyze the association between prospectively collected RR values and kidney graft outcome in a substudy of the MP Trial.

METHODS

Study design

Study data were prospectively collected in the MP arm of the MP Trial. This randomized controlled trial compared MP with static cold storage for the development of DGF in all types of deceased donor kidneys within part of Eurotransplant: Belgium, the Netherlands, and the federal state of North Rhine-Westphalia in Germany. The MP Trial showed that MP significantly reduces the incidence of DGF (adjusted odds ratio (OR) 0.57 (0.36–0.88), P = 0.01) and increases one-year graft survival (hazard ratio 0.52 (0.29–0.93), P = 0.03).[48]

Briefly, all kidneys from eligible consecutive deceased donors (SCD, ECD, and DCD) aged 16 years or older, were included. We defined ECD kidneys according to criteria of the United Network for Organ Sharing (UNOS).[12] Among the DCD kidneys, only those originating from Maastricht category III donors were included.[13] One kidney from each donor was assigned to MP and the contralateral kidney to static cold storage according to regional and computer generated randomization lists. When a reliable connection to the perfusion machine was impaired either by an aortic patch that was too small or by too many renal arteries, randomization for this kidney pair was changed and the preservation methods switched. Kidneys were allocated according to standard Eurotransplant allocation rules without revealing the preservation method at the time of organ offer. A strictly paired design was maintained, in which both kidneys from one donor needed to be transplanted into different recipients. Both kidneys of a pair were excluded when one or both recipients died within 1 week after transplantation. For this analysis, only the data prospectively collected in the MP arm of the MP Trial were used. Informed consent from recipients was not required, as kidneys were randomized before organ allocation. Ethical approval was obtained from the Eurotransplant Ethical Advisory Committee, the Kidney Advisory Committee, and ethics review boards in each trial region.

Preservation method

Kidneys were flushed *in situ* with the University of Wisconsin solution (64%) or histidine-tryptophan-ketoglutarate (32%); in 4% of cases the flush solution was not reported. Pulsatile MP was provided by LifePort® Kidney Transporter machines (Organ Recovery Systems, Itasca, IL, USA). Perfusion was started immediately after organ recovery, and was continued until transplantation. All kidneys were perfused with Belzer's machine perfusion solution, available as Kidney Preservation Solution-1® (1–8°C).[108] Perfusion was started immediately after organ recovery and was continued until transplantation. The systolic perfusion pressure was set at 30 mmHg and the machine continuously recorded the perfusion parameters. The LifePort® was used as a stand-alone preservation technique: changing the perfusion pressure or adding pharmacologic agents to the perfusion solution was not allowed. The MP kidney was transported to the recipient hospital without any monitoring. The transplantation team was blinded to the perfusion parameters. Because the transplantation team was blinded to

the perfusion parameters, the decision to accept or reject a kidney could not be biased by these parameters. The RR data were downloaded by the perfusionist at the end of MP, to be evaluated at a later time.

Follow-up

Recipients provided follow-up data to a secure online Eurotransplant database and were financially compensated to ensure maximal data completeness. No relevant irregularities were found during an external audit of a random sample of 10% of all patient follow-up data.

Study endpoints

DGF was defined as the need for dialysis in the first week after transplantation, preceding return of graft function. PNF was defined as the permanent lack of graft function. Death censored graft survival was the outcome measure for graft performance until 1 year posttransplantation. Because the LifePort® software calculates RR every 10 s (mmHg/mL/min), we chose to analyze RR data at 30 minutes, 1, 2 and 4 h and at the end of MP. These time points were chosen before disclosure of RR values.

Statistical methods

We performed univariable logistic regression analysis for DGF and then constructed a multivariable logistic regression model to find independent risk factors for DGF. The RR was entered as a covariate in these models. Other covariates were prespecified in the protocol before the MP Trial started. Because of a limited number of events in this subgroup analysis we only included those prespecified factors that were significantly associated with DGF in the MP Trial: cold ischemic time, donor type (donation after brain death [DBD] vs. DCD), donor age, retransplantation vs. first transplantation, and duration of pretransplant dialysis.[48] A receiver operator characteristic (ROC) curve was constructed to investigate the predictive accuracy of RR for DGF. Because the number of PNF was low, an association between PNF and RR could not be studied and PNF cases were excluded from further analysis. We performed unadjusted and adjusted Cox regression analysis for 1-year graft failure. Because of the low number of graft losses, we could only correct for two variables; we therefore chose RR, the variable of interest and donor age, the variable that was the strongest independent risk factor for graft failure in the MP Trial. To exclude a potential bias introduced by kidneys in which randomization needed to be switched, the logistic and Cox regression analyses only included kidneys that were randomized to and effectively preserved by MP.

Continuous variables are expressed as median and range, categorical variables as number and percentage. Two-sided P-values ≤0.05 were considered to indicate statistical significance. Endpoint interim analyses were not performed. All data analyses were performed using SPSS, SAS and R software.

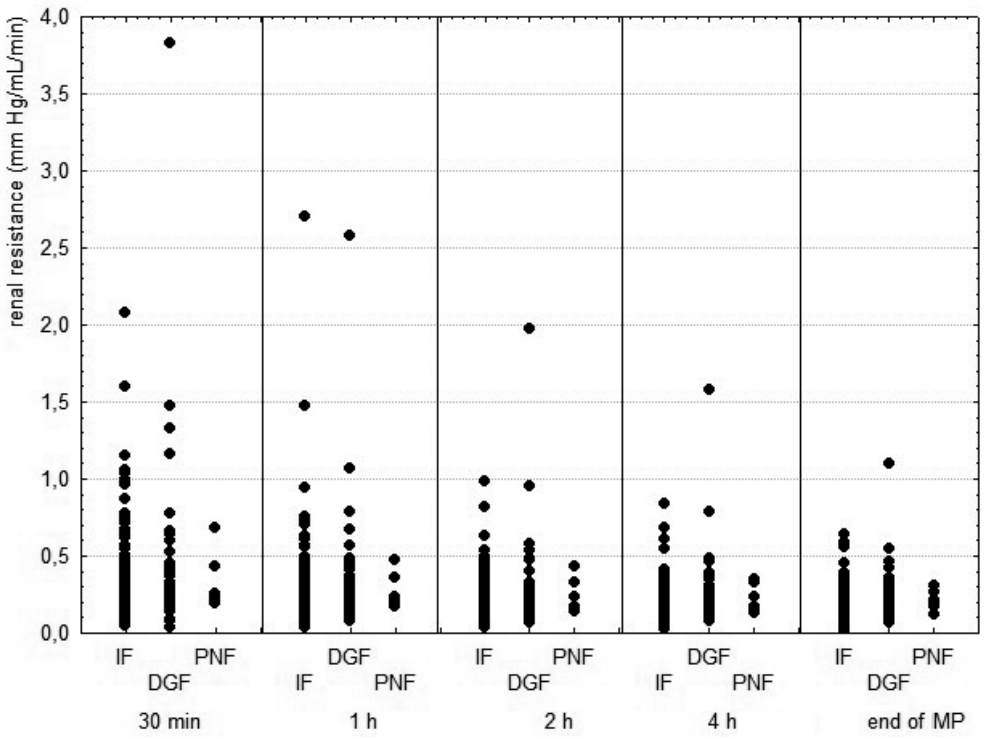

Figure 1: Dot plot representing the individual renal resistance values in function of perfusion time during hypothermic machine perfusion and early graft function. PNF: primary non-function defined as permanent lack of graft function (n = 6); DGF: delayed graft function defined as dialysis in the first week after transplantation, preceding return of graft function (n = 63); IF: immediate function (n = 257); MP: hypothermic machine perfusion.

RESULTS

Three hundred thirty-six deceased donor kidneys were preserved by MP between November 1, 2005 and October 31, 2006. We included 42 DCD kidneys (13%) and 294 DBD kidneys (87%), of which 203 were SCD and 91 were ECD. Table 1 shows donor and recipient characteristics, early graft function and 1-year graft and patient survival. Overall, 19% of machine perfused kidneys developed DGF; PNF occurred in seven cases (2%). The incidence of DGF was highest in DCD kidneys; PNF did not differ between SCD, ECD and DCD kidneys. One-year patient and death censored graft survival was 97% and 94% and comparable between all donor types. The RR data were available in 326 cases (Figure 1 and Table 2). The RR of PNF kidneys were intermediate between RR of DGF and immediately functioning kidneys. Because of the low number of events, these PNF cases were excluded from further analyses. Randomization was switched in 24 donors (7%) because of aberrant vascular anatomy, making the connection to the LifePort® difficult. Switching randomization had no significant effect on the incidence

of DGF; of these 24 kidneys, 8 developed DGF whereas 16 had immediate graft function (P = 0.12). To avoid an impact on the outcome of the logistic and Cox regression models, these 24 kidneys were excluded from the regression analyses.

Variable	All (n = 336)	SCD (n = 203)	ECD (n = 91)	DCD (n = 42)	P-value
Donor characteristics					
Age[1] (year)	51 (16–81)	46 (16–59)	66 (50–81)	42 (17–60)	0.001[2]
Cold ischemic time[1] (h)	15 (3–30)	15 (5–30)	13 (3–23)	16 (4–25)	0.001[2]
Recipient characteristics					
Age[1] (year)	53 (11–79)	51 (11–76)	62 (20–79)	50 (24–69)	0.001[2]
Duration pretransplant dialysis[1] (year)	4.5 (0.2–18)	4.5 (0.2–14)	4.7 (0.4–11)	4.7 (1.1–18)	0.11[2]
Early graft function, n (%)					
Immediate function	266 (79)	178 (87)	68 (75)	20 (48)	0.001[2]
Primary nonfunction[1]	7 (2)	3 (1)	23 (3)	1 (2)	0.61[2]
Delayed graft function[3]	63 (19)	22 (11)	20 (22)	21 (50)	0.001[2]
Graft survival, n (%)					
Death censored					
At 3 months	320 (95)	194 (96)	85 (93)	41 (98)	0.61[4]
At 6 months	317 (94)	193 (95)	84 (92)	40 (95)	0.61[4]
At 1 year	317 (94)	193 (95)	84 (92)	40 (95)	0.61[4]
Including patient death					
At 3 months	319 (92)	194 (96)	84 (92)	41 (98)	0.35[4]
At 6 months	314 (94)	192 (95)	82 (90)	40 (95)	0.32[4]
At 1 year	309 (92)	189 (93)	80 (88)	40 (95)	0.22[4]
Patient death, n (%)					
At 3 months	1 (0.3)	0 (0)	1 (1)	0 (0)	–
At 6 months	6 (2)	2 (1)	4 (4)	0 (0)	0.08[4]
At 1 year	11 (3)	5 (2)	6 (7)	0 (0)	0.08[4]

Table 1: Population characteristics, early graft function, 1-year patient and graft survival of machine-perfused kidneys in the Machine Preservation Trial. All values of donor and recipient characteristics are median (range). SCD = standard criteria donor; ECD = extended criteria donor as defined by UNOS criteria; DCD = donation after cardiac death = Maastricht category III.
[1] Primary non function: permanent lack of allograft function.
[2] Wald test.
[3] Delayed graft function: need for dialysis in the first week after transplantation.
[4] Log-rank test.

Univariate analysis showed that RR was a risk factor for the development of DGF at 30 minutes, 2 and 4 h and at the end of MP (Table 3). In multivariate analysis, only RR at the end of MP proved to be an independent risk factor of DGF in addition to donor type (DBD vs. DCD), donor age and retransplantation (Table 4). The RR data at 4 h showed a trend towards significance in the multivariate analysis for the risk of DGF (OR 9.68 (0.79–118.39); P = 0.076). The c-statistic of the ROC curve for RR at the end of MP was 0.58. The RR was also a risk factor for 1-year graft failure in both unadjusted and adjusted Cox regression analysis (Tables 3 and 4).

Variable	RR at 30 min MP	RR at 1 h MP	RR at 2 h MP	RR at 4 h MP	RR at end MP
All	0.28	0.22	0.20	0.18	0.17
	(0.04–3.83)	(0.04–2.70)	(0.04–1.97)	(0.03–1.58)	(0.02–1.10)
	n = 325	n = 324	n = 323	n = 302	n = 325
Donor type					
SCD	0.25	0.21	0.19	0.18	0.16
	(0.05–3.83)	(0.04–2.70)	(0.04–1.97)	(0.03–1.58)	(0.02–1.10)
	n = 202	n = 202	n = 202	n = 193	n = 202
ECD	0.26	0.23	0.21	0.20	0.18
	(0.04–1.47)	(0.07–0.79)	(0.06–0.66)	(0.05–0.57)	(0.03–0.51)
	n = 82	n = 81	n = 80	n = 69	n = 82
DCD	0.29	0.23	0.21	0.20	0.18
	(0.08–3.4)	(0.09–2.62)	(0.09–1.72)	(0.08–0.79)	(0.07–0.88)
	n = 41	n = 41	n = 41	n = 40	n = 41
Early graft function					
IF	0.24	0.21	0.19	0.17	0.16
	(0.05–2.08)	(0.04–2.7)	(0.04–0.99)	(0.03–0.84)	(0.02–0.64)
	n = 257	n = 256	n = 254	n = 234	n = 256
DGF	0.28	0.23	0.21	0.20	0.18
	(0.04–3.83)	(0.08–2.58)	(0.07–1.97)	(0.08–1.58)	(0.07–1.10)
	n = 62	n = 62	n = 63	n = 62	n = 63
PNF	0.25	0.22	0.21	0.21	0.20
	(0.12–0.68)	(0.17–0.47)	(0.14–0.43)	(0.11–0.35)	(0.12–0.31)
	n = 6	n = 6	n = 6	n = 6	n = 6

Table 2: Renal resistance of machine-perfused kidneys in function of donor type and early graft function
All values are median (range). RR = renal resistance in mm Hg/mL/min; SCD = standard criteria donor; ECD = extended criteria donor as defined by UNOS criteria; DCD = donation after cardiac death = Maastricht category III; IF = immediate function; DGF = delayed graft function defined as dialysis in the first week after transplantation, preceding the return of graft function; PNF = primary non function defined as permanent lack of graft function; MP = hypothermic machine perfusion.

Variable	Odds ratio (95% CI)	Hazard ratio (95% CI)	P-value
Delayed graft function			
RR at 30 min of MP	2.41 (1.02–5.71)		0.046
RR at 1 h of MP	2.28 (0.86–6.04)		0.097
RR at 2 h of MP	8.88 (1.39–56.59)		0.021
RR at 4 h of MP	16.30 (1.67–158.95)		0.016
RR at end of MP	44.43 (2.79–706.79)		0.007
Graft failure			
RR at end of MP		12.970 (1.20–140.74)	0.035

Table 3: Univariable analysis for delayed graft function and 1-year graft failure in 302 machine-perfused kidneys. Logistic regression model for delayed graft function and Cox regression model for graft failure. CI = confidence interval; RR = renal resistance expressed in mm Hg/mL/min; MP = hypothermic machine perfusion.

Variable	Odds ratio (95% CI)	Hazard ratio (95% CI)	P-value
Delayed graft function			
RR at end of MP (mmHg/mL/min)	38.1 (1.56–934)		0.026
Donor age (year)	1.03 (1.00–1.06)		0.036
Donor type[1]	0.10 (0.04–0.25)		< 0.0001
Retransplant versus first transplant	2.29 (1.37–3.83)		0.002
Duration of pretransplant dialysis (year)	1.07 (1.00–1.16)		0.065
Cold ischemic time (h)	1.05 (0.90–1.24)		0.57
Graft failure			
RR at end of MP (mmHg/mL/min)		12.33 (1.11–136.85)	0.004
Donor age (year)		1.01 (0.98–1.05)	0.5

Table 4: Multivariable risk analysis for delayed graft function and 1-year graft failure in 302 machine-perfused kidneys. Logistic regression model for delayed graft function and Cox regression model for graft failure. CI = confidence interval; RR = renal resistance; MP = hypothermic machine perfusion.
[1] Donor type was stratified to either donation after brain death (standard criteria donors and extended criteria donors) or donation after cardiac death.

DISCUSSION

This analysis of prospectively collected RR values of kidneys stored by MP showed that RR is an independent risk factor for both DGF and 1-year graft failure. These findings suggest that RR is an important additional objective tool to be used in kidney graft quality assessment. Nevertheless, our analysis also indicates that RR, given its low predictive accuracy, cannot be used as a stand-alone viability parameter to accept or discard a given kidney, unlike current practice in some centers.

We showed that RR at the end of MP was an independent risk factor for the later development of DGF. A potentially important benefit of RR could therefore be the ability to estimate the risk of a particular kidney to develop DGF. This information may help clinicians in the postoperative management of their patients (e.g. delaying or lowering exposure to calcineurin inhibitors, additional information to institute dialysis, etc.). The RR at the end of MP is not a fixed point in time but depends on the duration of MP. Knowing the risk profile of a particular kidney earlier in the preservation process might be of greater benefit, because it would provide a time window necessary for selecting a particular recipient for a particular kidney. In fact, RR data at 4 h showed a trend towards significance in the multivariable analysis for the risk of DGF and RR values remained stable after 4 h until the end of MP.

Although it would be appealing to use the RR value as a stand-alone parameter to assess the risk of DGF, we found that the c-statistic of the ROC curve of RR for predicting DGF was 0.58. This c-statistic implies that any determined RR threshold would result in a relatively poor predictive capacity for DGF. To illustrate this, we attempted a post hoc analysis to define a RR threshold value in our data set. The calculated discriminative capacity of this threshold (RR = 0.28 mmHg/mL/min) was weak (specificity 93%, sensitivity 17%, positive predictive value 40% and negative predictive value 81%). These results are not surprising given the multifactorial nature of the pathogenesis of DGF. Several donor, procurement and recipient-related risk factors (age, donor type, warm and cold ischemic time, inotropy, hypertension,

hypovolemia, number of previous transplants, etc.) influence DGF[31] and it would be too simplistic to believe that one single new risk factor, RR, would replace all the others.

Our observations are in concordance with previous reports that recommend caution in using RR in the assessment of kidney quality. Indeed, Sonnenday *et al*. stressed the importance of considering not only the perfusion parameters but all donor factors when assessing graft quality. These authors could successfully transplant 11 of 14 kidneys with favorable donor characteristics that had been turned down by other centers due to "poor" perfusion parameters.[192] Mozes *et al*. analyzed 336 consecutive machine-perfused ECD kidneys and showed that the outcome of kidneys with "poor" perfusion parameters (0.40 mmHg/mL/min < RR < 0.60 mmHg/mL/min) was similar to the kidneys with "good" perfusion parameters.[193] More recently, Guarrera *et al*. reported acceptable short- and long-term results in a small series of deceased donor kidneys with "poor" perfusion parameters (flow <80 mL/min/100g and RR >0.40 mmHg/mL/min/100g) but no other high donor risk factors.[194]

The necessity to cautiously interpret RR data is also illustrated by the unexpected finding that all the PNF cases in our cohort had RR values intermediate between functioning and DGF kidneys. Because only seven cases of PNF were encountered, statistically sound conclusions regarding a possible association between RR and PNF could not be drawn. However, it is remarkable that when RR criteria, commonly used to discard kidneys likely to fail (RR >0.40 mmHg/mL/min),[193] were to be applied in our study population, no single PNF case would have been prevented, but eight viable kidneys (2.5%) would have been erroneously discarded (four kidneys with immediate function, four with DGF).

Importantly, we also found that RR is a risk factor for 1-year graft failure. As there were only 18 graft losses, we could only correct for one additional factor, donor age. Nevertheless, our observation is in line with a recent retrospective analysis of 454 preselected MP kidneys in the donor service area of New York showing that a RR >0.3 mmHg/mL/min at 3 and 5 h of MP is a significant predictor of 1-year graft survival in Cox regression analysis.[195] In analogy with the carotid intima–media thickness that reflects a person's cardiovascular risk profile,[196] we hypothesize that RR of perfused kidneys may reflect their intrinsic morphological "quality" and subsequent likelihood of successful outcome after kidney transplantation. Correlation studies between RR and renal histology parameters are warranted to determine which particular morphological features of the kidney graft are mirrored by RR. In comparison to single biopsies, that are subject to sampling error and interobserver variability, RR may reflect the overall quality of a given kidney better.

Although our study clearly shows that RR is an independent risk factor for both DGF and graft failure, the determinants of transplant outcome are multifactorial and it remains elusive to predict outcome based on RR data (or other parameters) alone. Numerous risk scores, implementing several independent donor, procurement and recipient risk factors have already been proposed. For example, Irish *et al*. have constructed a "composite DGF score" that has a moderate predictive power for DGF (c-statistic 0.70).[197,198] Rao *et al*. recently developed the kidney donor risk index to estimate the risk of graft failure.[182] Adding RR to such risk

scores will likely increase their predictive accuracy and provide better tools to evaluate kidney quality. Another parameter that may also improve the predictive value of these multifactorial scoring systems is the concentration of certain biomarkers in the perfusate because, like the RR, they have been shown to independently correlate with DGF.[199]

The aforementioned data on the impact of RR on transplant outcome apply to all deceased donors with the exception of uncontrolled DCD donors (Maastricht category I, II). Such donors were not included in our trial. In most centers within Eurotransplant, kidneys recovered from uncontrolled DCD donors are already routinely preserved by MP and when designing the study, it was felt unethical to randomize these kidneys to static cold storage because of their particularly high risk of PNF (up to 13.5% for category II kidneys).[200] The exclusion of uncontrolled DCD donors may account, at least in part, for the low incidence of PNF in our study.

A potential bias in our study is the change of randomization in 25 cases because of vascular anomalies of a right or left kidney that prevented connection to the machine perfusion device. In these cases, the other kidney was machine perfused. This could have led to the exclusion of kidneys with a higher risk of DGF. Vascular anomalies had no significant effect on the development of DGF in our trial. However, to minimize a possible bias, we performed the logistic and Cox regression analyses only with kidneys that were allocated to and effectively underwent MP.

An important technical point for the interpretation of our data and their possible application in the clinics is that all kidneys were perfused with LifePort® machines, whereas many previously reported studies used different systems, among which the RM3 machine (Waters Medical Systems, Rochester, MN, USA). This is noteworthy because the LifePort® uses a pressure controlled roller pump to deliver the perfusate, creating sinusoidal flow curves, whereas the RM3 has a flow controlled pumping system. This gives rise to different wave pressure forms and different calculated RR values. Although absolute RR values calculated by the two devices cannot be compared directly, the association of RR and DGF/1-year graft failure found in our analysis remains valid since only one pump type was used. We believe that similar conclusions would have been reached if another system had been used, albeit probably with different RR values.

In conclusion, this study shows that RR during MP of all common types of deceased donor kidneys is an independent risk factor for the development of DGF and for 1-year graft failure. Therefore, RR represents an additional and objective source of information that can assist clinicians in their decision making process. However, DGF and graft failure have a complex pathogenesis and cannot be predicted with precision based on RR as a stand-alone assessment tool. More accurate prediction of graft outcome will require integration of perfusion parameters into multifactorial graft quality scoring systems.

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Chapter 12

General discussion and perspective

The vast majority of scientific research in the field of kidney transplantation tends to be focused on the recipient. A quick PubMed search on February 8, 2011 yielded approximately 87,000 articles on the topic "kidney transplantation", of which almost 40,000 include the word "immunosuppression", or "immunosuppressive" in the abstract. Only 2,300 papers (2.6%) deal with donor management, and organ preservation is the topic of no more than 3,600 studies in kidney transplantation (4.1%). The same pattern can be observed at the average organ transplantation conference, where donor management and organ preservation related sessions are usually scarce, attended by few people, and situated in a small room at the far end of the convention center. Immunosuppression and -modulation, as well as other aspects of recipient management offer practically unlimited clinical and pre-clinical research opportunities. Without modern pharmacologic recipient management, kidney transplantation outcome as we know it would not be possible. Hence, it remains important to continuously seek for improvement of posttransplant protocols, with a main focus on new drugs, novel combinations of pharmacologic regimens, and other interventions that will modulate the host immune response in order to prevent acute rejection and chronic allograft nephropathy. However, the studies in this thesis illustrate that various donor, donor management, and organ preservation related factors have a profound impact on outcome after renal transplantation. Many of these factors can be influenced in such a way that posttransplant results will improve significantly. Apart from the interventions that are studied in this thesis, one very important factor is cold ischemic time. The multivariate models in chapters 3 and 5 show that increased cold ischemic time is significantly and independently associated with an elevated risk of delayed graft function and graft failure. The American Organ Procurement and Transplantation Network (OPTN) database can be utilized to obtain additional detailed information on the association beween cold ischemic time and posttransplant outcome. When the cohort of deceased donor single kidney recipients between 1994 and 2007 (n=99,860) is studied, all important end points after transplantation, including acute rejection and graft survival, are strongly influenced by a few hours rise in cold ischemic time above 8 hours (Figure 1a-e). These figures show that the usual assumption that a deceased donor kidney is safe as long as cold ischemic time stays below 18-24 hours is incorrect. Deceased donor kidneys should always be transplanted as soon as possible, for a few hours reduction in cold ischemic time will result in a significant and clinically relevant improvement in outcome. In the following section a reference example is outlined:

In 2007 the large (n=1,645) prospective multicenter Symphony study compared various posttransplant immunosuppressive protocols, among which a regimen consisting of low-dose ciclosporin combined with daclizumab (an anti-CD25 antibody) induction therapy, and a regimen with standard-dose ciclosporin without daclizumab. Recipients in the low-dose tacrolimus + daclizumab group had a 3.8% better 1-year graft survival, a 1.8% lower incidence of acute rejection, and a creatinine clearance at 1 year which was 2.3 ml/min higher (relative increase 4%) compared to patients in the standard-dose ciclosporin group without daclizumab.[201] The aforementioned data in the OPTN database suggest that reduction

of the average cold ischemic time with just a few hours is likely to result in an improved postttansplant outcome which is in the same order as the effect that low-dose ciclosporin + daclizumab had versus standard-dose ciclosporin. A standard induction regimen with daclizumab will cost approximately € 5,000 extra per patient. This example illustrates that slightly speeding up organ transport, crossmatch, and operating room logistics might result in a similar improvement in outcome as a costly new immunosuppressive drug will achieve.

The retrospective study presented in chapter 3 shows that donor age has a large impact on delayed graft function and graft survival after kidney transplantation. This study, as well as multivariate models in chapters 5, 6, and 7 suggest that donor age is probably the strongest determinant of posttransplant outcome. The average organ donor today is older than donors were a few decades ago. Although donor age itself cannot be influenced, studies within Eurotransplant have suggested that the implementation of an old-for-old allocation policy could make best use of older donor kidneys, by allocating these grafts to older recipients who have a shorter life expectancy. In theory, even a renal graft recovered from an older donor will often outlive its senior recipient, thus reducing death censored graft failure in this group of aged patients. The papers that report results from this Eurotransplant Senior Program (ESP) conclude that graft and patient survival were not negatively affected compared to standard allocation and that, therefore, old-for-old allocation is an effective system.[78,85] However, it can be argued that these reports emphasize the wrong graft survival data. When looking at the group of older kidneys, indeed no significantly different 6-year graft and patient survival was observed between old-to-old and old-to-any allocation. This is in line with the theoretical assumption outlined above. If not the group of older kidneys, but the group of older recipients is studied, there appears to be a rather large difference in graft and patient survival between old-to-old and any-to-old allocation in favor of the latter policy, both with and without censoring for death with a functioning graft. This implies that with regard to patient and graft survival, older *recipients* as a group are far better off with standard allocation than with old-for-old allocation. The finding that older *kidneys* perform equally well in older recipients as in a group with recipients of all ages is interesting, but less relevant. Exactly these associations were also observed in the old-for-old simulations in chapter 3, along with the inevitable effect that younger recipients as a group will do slightly better when old-for-old allocation is implemented, since this group will then receive on average younger (i.e. higher quality) kidneys. Another important argument in favor of old-for-old allocation is that it will reduce waiting time for older transplant candidates. However, it is questionable whether this objective justifies the practice of systematically transplanting inferior-quality organs into older patients. Indeed, even with the presumably shorter waiting time in old-for-old allocation, patient survival was inferior compared to older recipients who received standard allocation (any-to-old). The shorter waiting time in the ESP and the associated shorter time on dialysis did apparently not outweigh the negative effect that the on average lower-quality kidney grafts had on patient survival. Therefore, old-for-old allocation seems to be much

effort for a net effect which is likely to be close to zero. In addition, serious ethical concerns apply to the deliberate shifting of graft and life years from one group of recipients to another.

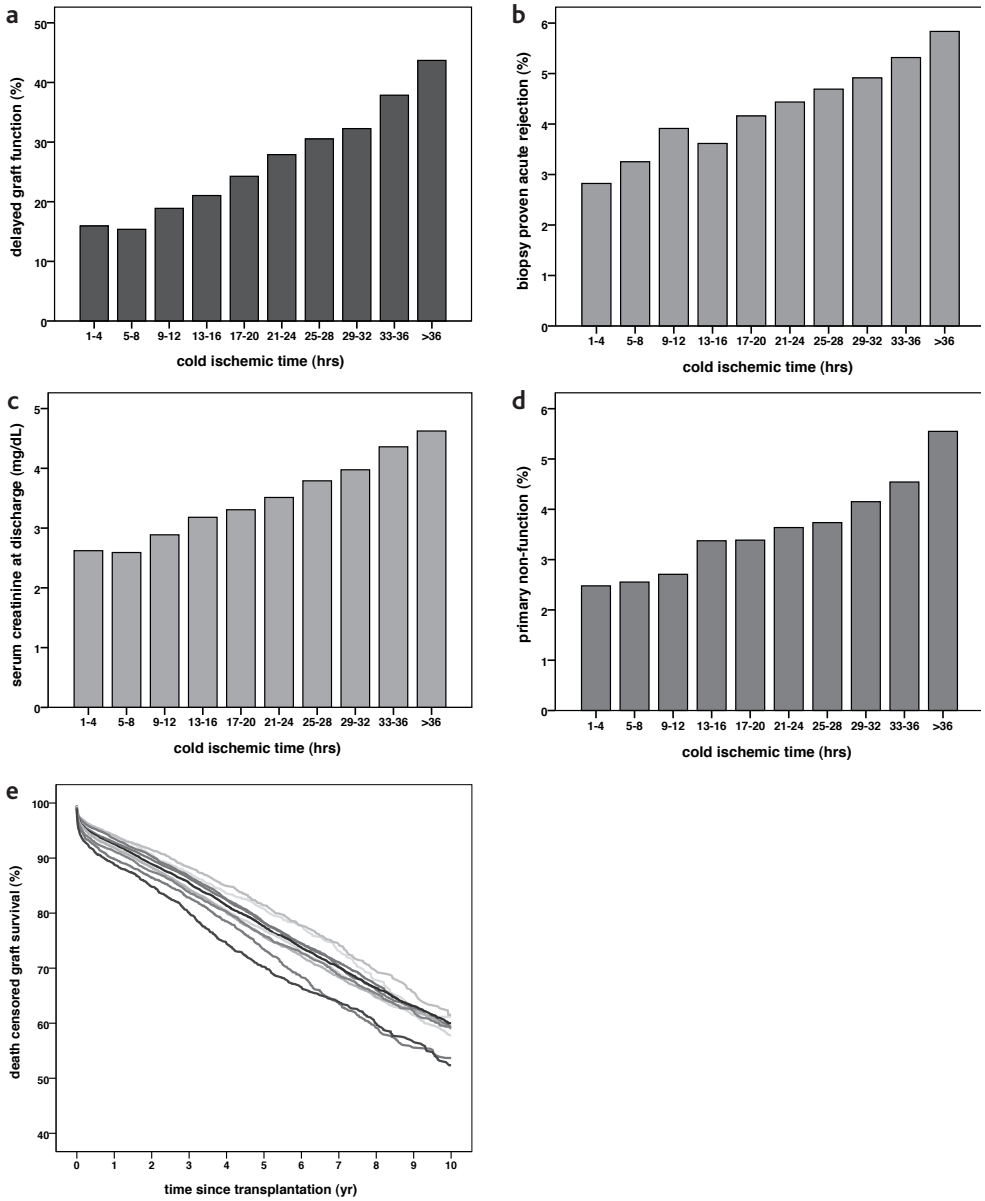

Figure 1: Delayed graft function (**a**), primary non-function (**b**), acute rejection (**c**), serum creatinine at discharge (**d**), and graft survival at 10 years posttransplant (**e**) as a function of cold ischemic time. In panel (e), cold ischemic time varies between 1 and 36 h and each curve below another curve represents a subsequent 4 h wide cold ischemic time category. Retrospective data derived from the Organ Procurement and Transplatation Network database, cohort 1994-2006, recipients of deceased donor single kidneys (n=99,860).

Normothermic recirculation of the donor's body before cold organ preservation is instituted has briefly appeared in the transplantation literature approximately 10 years ago.[39,93,98] But after these publications, no other reports on results of the method have been published. In 2010, the group from the Hospital Clínic in Barcelona, who have developed the method, published an article in which an update was given on normothermic recirculation results in the last decade. Their data show a 60% graft survival and a 77% patient survival at 3 years after liver transplantation. Recent figures with regard to kidney transplantation after normothermic recirculation are not shown. In addition, the group reports a high incidence of ischemic biliary tract complications, and a very high liver discard rate of 75% after uncontrolled Maastricht category II donation after cardiac death.[92] The authors conclude that not normothermic recirculation, but normothermic machine perfusion during the whole interval between organ procurement and transplantation is likely to be the most promising method for the future of kidney and liver transplantation from uncontrolled donors after cardiac death. Indeed, normothermic machine perfusion of kidney and liver grafts has been shown to be superior to hypothermic machine perfusion and static cold storage in preclinical studies.[202-204] Theoretically, the superiority of normothermia over hypothermia as the main principle in organ preservation is not surprising. Mimicking human physiology and continuously providing an organ with its metabolic needs is likely to result in a better preserved graft than the usual cascade which consists of warm ischemia, followed by cold ischemia, followed by warm reperfusion in the recipient. However, the equipment needed for normothermic machine perfusion is complex and requires intensive monitoring, which makes transport logistics rather cumbersome.[38] Moreover, equipment failure is an emergency situation which results in warm ischemia of the graft and requires direct intervention. Unaccompanied transport of a normothermic machine perfusion device will never be a realistic option. In contrast, when hypothermic machine perfusion fails, the kidney remains safely cold stored inside the perfusion machine. Hence, the latter method is more suitable for stand-alone operation and travel.

A brief period of normothermic recirculation before organ recovery would theoretically combine the beneficial effect of physiological oxygenized perfusion directly after warm ischemia with the logistically more feasible method of hypothermia during organ transport. The animal study presented in chapter 4 revisits normothermic recirculation in kidney transplantation and addresses the question whether the method can indeed mitigate warm ischemic injury. Unfortunately, no such effect was found. Despite various relevant limitations of this preclinical study, the data do not encourage a prospective study of normothermic recirculation in human donors after cardiac death. The total silence in the published literature on the topic of normothermic recirculation in clinical renal transplantation that now lasts for more than a decade perhaps reflects the fact that, so far, no consistently beneficial effect of the method has been shown for kidneys. Positive experiences in the clinic are repeatedly reported at conferences by the Barcelona, Madrid, and St. Petersburg groups, but so far no comparative clinical study has been conducted to test the presumed positive effect of

normothermic recirculation on kidneys. A sufficiently powered prospective clinical study will be the only way to answer the question whether normothermic recirculation should have any role in management of kidney donors after cardiac death.

Hypothermic machine perfusion is by no means a novel kidney preservation method. In fact, some of the first kidney transplants ever were performed after the organ had been machine perfused.[91,205] Static cold storage was widely adopted in the 1980s, when improved synthetic organ preservation fluids such as the University of Wisconsin solution became available and Opelz and Terasaki published a retrospective report which suggested that cold storage was superior to machine perfusion.[103,132] Until one decade ago, only a few centers persisted to use machine perfusion for selected marginal donor kidneys. Several retrospective analyses published shortly after the 1990s suggested that, with the average donor kidney being more marginal than back in the 1980s, hypothermic machine perfusion may nowadays be superior to cold storage of deceased donor kidneys.[105,106] However, an adequately powered RCT was needed to resolve the controversy and show whether machine perfusion has any advantage over static storage. The Machine Preservation Trial, the results of which are reported in chapters 5 through 11, showed that machine perfusion should be the method of choice for the preservation of all common types of deceased donor kidneys. The formal subgroup analyses in chapter 5 demonstrated that the magnitude of the beneficial effect of machine perfusion in terms of delayed graft function reduction is similar for kidneys recovered from standard criteria donors, expanded criteria donors, and donors after cardiac death. These findings are reinforced by the two separate pre-specified sub-studies in chapters 6 and 7, which focused in detail on the effect of machine perfusion versus cold storage in kidneys derived from brain dead expanded criteria donors and donors after cardiac death.

Our 3-year follow-up analysis confirms that machine perfusion will lead to a superior graft survival for kidneys donated after brain death, especially in those renal grafts recovered from expanded criteria donors. In addition, this analysis shows that kidneys procured from donors after cardiocirculatory death do not benefit from machine perfusion in terms of a better 3-year graft survival. Machine perfusion will lead to a substantial reduction in delayed graft function for such kidneys, and therefore sufficient rationale remains for machine perfusing kidneys from donors after cardiocirculatory death. Several studies in this thesis have shown that delayed graft function could be an important risk factor for graft failure, but our data also suggest that this association does not apply to kidneys donated after cardiocirculatory death in the same magnitude as it does to kidneys recovered from brain dead donors. The substantial reduction in delayed graft function due to machine perfusion does apparently not lead to a lower risk of graft failure for this subgroup of renal grafts. Mechanistic studies into the precise nature of delayed graft function are needed to determine whether kidneys derived from donation after cardiocirculatory death exhibit a different, perhaps less detrimental, type of delayed graft function compared to grafts recovered from donation after brain death. Part of the explanation of this phenomenon may be that, in a brain dead donor, renal grafts are

subjected to the detrimental pro-inflammatory and pro-coagulatory effects of brain stem death.[11,206] Hence, delayed graft function in these kidneys may have a more immunologic cause, in contrast to kidneys procured from donors after cardiocirculatory death. In the latter group, delayed graft function could be primarily a symptom of tubular necrosis due to ischemic injury, from which a kidney will be more likely to make a good recovery.

A parallel British RCT, the PPART study, failed to show superiority of machine perfusion over cold storage for the preservation of kidneys donated after cardiac death and reported no difference in delayed graft function between machine perfused and cold stored kidneys. However, this study had a rather uncommon sequential design, in which the chances of obtaining statistically significant results were determined at regular intervals during the enrollment period. After inclusion of no more than 45 kidney pairs, the study was stopped prematurely. Statistical models had indicated that further inclusion was unlikely to result in a significantly different incidence of delayed graft function between the machine perfusion and the cold storage arm.[138] Differences between the British and our study may explain the discrepancy in results. Of 25 centers, only five participated in the UK trial and most kidneys were transplanted locally. In contrast, our study on kidneys donated after cardiac death was fully integrated in everyday practice of an international organ sharing organization. Centers were blinded at organ offer, which might not have been the case in the British trial as the same unit often performed organ recovery and transplantation. We started machine perfusion immediately after procurement, whereas this was frequently delayed in the British trial. It may be that pumping kidneys throughout the entire preservation period is necessary for kidneys to fully benefit from machine perfusion.[207] Finally, the novel methodology that was utilized in the British study has never before been tested in a similar RCT setting. It remains unclear whether the stopping rules that were enforced by this complex method truly reflect that finding statistically significant differences cannot be expected, had a sufficiently powered complete data set been obtained.

In addition to the favorable clinical results that the Machine Preservation Trial found, the cost-effectiveness analysis in chapter 9 confirms that machine perfusion should be adopted for all types of deceased donor kidneys. Its implementation would result in less delayed graft function, an improved 1-year graft survival, and lower overall costs compared to cold storage. With this robust evidence in place, there can be no reason but political issues to refrain from broad implementation of hypothermic machine perfusion in Eurotransplant and other organ exchange organizations. Unfortunately, an important appraisal of the clinical utility and cost-effectiveness of machine perfusion versus cold storage by the British National Institute for Health and Clinical Excellence (NICE) had a rigid closing date a few months before the final results of the Machine Preservation Trial became available.[208] NICE based its recommendations in part on a very detailed cost-effectiveness study by Bond *et al*, which was also conducted too early to take the final results of our studies into account.[47] Both the NICE report and the publication by Bond *et al*, which are largely based on retrospective

evidence and on the prematurely stopped PPART study, suggest that insufficient evidence is available to recommend machine perfusion as the standard method for deceased donor kidney preservation. Although these reports are comprehensive and well nuanced, the structural omission of final data from our studies has rendered them outdated directly after their publication.

How exactly the logistics of machine perfusion should be organized remains to be determined. In contrast to the situation in the United States, where organ exchange is centralized around organ procurement organizations and most kidneys stay in the region of origin, in Eurotransplant long-distance international exchange is common and therefore renal machine perfusion has to be organized with stand-alone machines that can be transported in the same fashion as the traditional cold storage ice box. A realistic scenario could be as follows: Connection of kidneys to the machine, as well as retrieval of organs from the machine is an easy task and is probably best executed by the procurement / transplant surgeon at the instruction of a trained transplant coordinator. In The Netherlands, each of the four procurement regions should have 4-6 perfusion machines, which the transplant coordinator can bring to the donor hospital. The recipient center will clean and keep the empty perfusion machine in its own stock. It can be expected that the number of machines per region will stay in equilibrium most of the time. In case of shortage, machines can be transported by courier between two regions. Alternative scenarios, including the establishment of independent perfusion centers which stock all machines and provide procurement regions with their services could also be feasible, depending on the available manpower among transplant coordinators and the reimbursement structure chosen.

Apart from its clinical effectiveness on posttransplant outcome, machine perfusion is often advocated for its presumed diagnostic potential. Numerous retrospective analyses have addressed the prognostic value of renal intravascular resistance and flow rates during machine perfusion, as well as measurement of biomarkers in machine perfusion perfusate.[105,106,152,153,173,191,193,209] All such retrospective analyses suffer from a certain amount of selection bias, since kidneys are discarded based on presumably unfavorable pump characteristics and/or biomarker concentrations. In addition, most kidneys have undergone a usually subjective selection process to determine which organ should be pumped. Therefore, only a prospective study in an unselected data set can give insight into the true prognostic potential of perfusate biomarkers and pump parameters. The studies in chapters 10 and 11 are based on such prospective and unselected data. Although our data did show some independent predictive value of renal resistance and three perfusate markers, the most important conclusion that can be drawn from these studies is that perfusate biomarkers and pump parameters should never be utilized as a sole trigger to accept or discard a donor kidney. Their only justified application seems to be to consider each marker as one of the many factors that determine posttransplant outcome, and to adjust recipient management

accordingly. It can be argued that the impact of biomarker measurement is only marginal and may not be worth the additional expense. Renal resistance can be determined without extra costs, and is therefore a more realistic predictive variable to be considered during machine perfusion.

In conclusion, the results brought forward by the studies in this thesis once again emphasize that improving kidney transplant outcome starts in the donor. Better methods for deceased donor management, as well as superior organ preservation techniques are likely to make a relevant difference for the recipient. Normothermic recirculation will have to be tested in a randomized clinical study before it can be implemented in everyday practise. Hypothermic machine perfusion has been proven superior to static cold storage and should be utilized for most types of deceased donor kidneys. Anticipating outcome by means of perfusate biomarkers and machine perfusion characteristics has to be be done with caution. However, when more physiological, normothermic preservation methods become available, viability assessment before transplantation could become a useful tool to predict posttransplant organ performance. This thesis shows that static cold storage for marginal donor kidneys belongs to the past, and that the present should be dominated by hypothermic machine perfusion. Normothermic preservation may have the future, and research in the field of donor management and organ preservation should focus on this topic in the next decade.

References

1. Abouna GM. The use of marginal-suboptimal donor organs: a practical solution for organ shortage. Ann Transplant 2004; 9:62-66.
2. Cohen B, Smits JM, Haase B et al. Expanding the donor pool to increase renal transplantation. Nephrol Dial Transplant 2005; 20:34-41.
3. Pessione F, Cohen S, Durand D et al. Multivariate analysis of donor risk factors for graft survival in kidney transplantation. Transplantation 2003; 75:361-367.
4. Collini A, De Bartolomeis C, Ruggieri G et al. Long-term outcome of renal transplantation from marginal donors. Transplant Proc 2006; 38:3398-3399.
5. Suthanthiran M, Strom TB. Renal transplantation. N Engl J Med 1994; 331:365-376.
6. Petechuk D. Organ transplantation. Westport, CT, USA: Greenwood Press; 2006.
7. Manley GT. Introduction: decompressive craniectomy for trauma and cerebrovascular disease. Neurosurg Focus 2009; 26:E1.
8. Merrill JP, Murray JE, Harrison JH et al. Successful homotransplantation of the human kidney between identical twins. J Am Med Assoc 1956; 160:277-282.
9. Halloran PF. Immunosuppressive drugs for kidney transplantation. N Engl J Med 2004; 351:2715-2729.
10. A definition of irreversible coma. Report of the Ad Hoc Committee of the Harvard Medical School to Examine the Definition of Brain Death. J Am Med Assoc 1968; 205:337-340.
11. Nijboer WN, Schuurs TA, van der Hoeven JA et al. Effect of brain death on gene expression and tissue activation in human donor kidneys. Transplantation 2004; 78:978-986.
12. Metzger RA, Delmonico FL, Feng S et al. Expanded criteria donors for kidney transplantation. Am J Transplant 2003; 3 Suppl 4:114-25.:114-125.
13. Kootstra G, Daemen JH, Oomen AP. Categories of non-heart-beating donors. Transplant Proc 1995; 27:2893-2894.
14. Hume D, Merrill J, Miller B et al. Experiences with renal homotransplantation in the human: report of nine cases. J Clin Invest 1955; 34:327-382.
15. Merrill J, Murray J, Takacs F et al. Successful transplantation of kidney from a human cadaver. J Am Med Assoc 1963; 185:347-353.
16. Voronoy YY. Sobre el bloqueo del aparato retículoendotelial del hombre en algunas formas de intoxicación por el sublimado y sobre la transplantación del riñón cadavérico como método de tratamiento de la anuria consecutiva a aquella intoxicación. El Siglo Méd 1936; 97:296.
17. Kootstra G, Kievit JK, Heineman E. The non heart-beating donor. Br Med Bull 1997; 53:844-853.
18. Cohen B, D'Amaro J, De Meester J et al. Changing patterns in organ donation in Eurotransplant, 1990-1994. Transpl Int 1997; 10:1-6.
19. Eurotransplant Annual Report, preliminary version. Eurotransplant International Foundation, Leiden. Oosterlee, A. and Rahmel, A. 379. 2005.
20. Davis CL, Delmonico FL. Living-donor kidney transplantation: a review of the current practices for the live donor. J Am Soc Nephrol 2005; 16:2098-2110.
21. Schemmer P, Mehrabi A, Friess H et al. Living related liver transplantation: the ultimate technique to expand the donor pool? Transplantation 2005; 80:S138-S141.

22. Kootstra G, Kievit J, Nederstigt A. Organ donors: heartbeating and non-heartbeating. World J Surg 2002; 26:181-184.
23. Ploeg RJ, Niesing J, Sieber-Rasch MH et al. Shortage of donation despite an adequate number of donors: a professional attitude? Transplantation 2003; 76:948-955.
24. Heineman E, Daemen JH, Kootstra G. Non-heart-beating donors: methods and techniques. Transplant Proc 1995; 27:2895-2896.
25. Dutch protocol for non-heart-beating organ donation. Available at http://www.transplantatiestichting.nl/cms/mediaobject.php?file=protocolnhb.pdf. Accessed 09-09-2009.
26. Critical Pathway for Donation After Cardiac Death. Available at http://www.unos.org/SharedContentDocuments/Critical_Pathway_DCD_Donor.pdf. Accessed 09-09-2009.
27. Suntharalingam C, Sharples L, Dudley C et al. Time to cardiac death after withdrawal of life-sustaining treatment in potential organ donors. Am J Transplant 2009; 9:2157-2165.
28. Reich DJ, Mulligan DC, Abt PL et al. ASTS Recommended Practice Guidelines for Controlled Donation after Cardiac Death Organ Procurement and Transplantation. Am J Transplant 2009.
29. Brook NR, White SA, Waller JR et al. Non-heart beating donor kidneys with delayed graft function have superior graft survival compared with conventional heart-beating donor kidneys that develop delayed graft function. Am J Transplant 2003; 3:614-618.
30. Biberthaler P, Luchting B, Massberg S et al. The influence of organ temperature on hepatic ischemia-reperfusion injury: a systematic analysis. Transplantation 2001; 72:1486-1490.
31. Perico N, Cattaneo D, Sayegh MH et al. Delayed graft function in kidney transplantation. Lancet 2004; 364:1814-1827.
32. Matzinger P. The danger model: a renewed sense of self. Science 2002; 296:301-305.
33. Boros P, Bromberg JS. New cellular and molecular immune pathways in ischemia/reperfusion injury. Am J Transplant 2006; 6:652-658.
34. Lemos FB, Ijzermans JN, Zondervan PE et al. Differential expression of heme oxygenase-1 and vascular endothelial growth factor in cadaveric and living donor kidneys after ischemia-reperfusion. J Am Soc Nephrol 2003; 14:3278-3287.
35. Bonventre JV. Dedifferentiation and proliferation of surviving epithelial cells in acute renal failure. J Am Soc Nephrol 2003; 14 Suppl 1:S55-61.:S55-S61.
36. Opelz G, Dohler B. Multicenter analysis of kidney preservation. Transplantation 2007; 83:247-253.
37. Brasile L, Stubenitsky BM, Haisch CE et al. Repair of damaged organs in vitro. Am J Transplant 2005; 5:300-306.
38. Reddy SP, Brockmann J, Friend PJ. Normothermic perfusion: a mini-review. Transplantation 2009; 87:631-632.
39. Fondevila C, Hessheimer AJ, Ruiz A et al. Liver transplant using donors after unexpected cardiac death: novel preservation protocol and acceptance criteria. Am J Transplant 2007; 7:1849-1855.
40. Moers C, Leuvenink HGD, Ploeg RJ. Non heart beating organ donation: overview and future perspectives. Transpl Int 2007; 20:567-575.
41. Bernat JL, D'Alessandro AM, Port FK et al. Report of a National Conference on Donation after cardiac death. Am J Transplant 2006; 6:281-291.

42. Bernat JL. The boundaries of organ donation after circulatory death. N Engl J Med 2008; 359:669-671.
43. Dejohn C, Zwischenberger JB. Ethical implications of extracorporeal interval support for organ retrieval (EISOR). ASAIO J 2006; 52:119-122.
44. Snoeijs MG, Dekkers AJ, Buurman WA et al. In situ preservation of kidneys from donors after cardiac death: results and complications. Ann Surg 2007; 246:844-852.
45. Alvarez J, del Barrio R, Arias J et al. Non-heart-beating donors from the streets: an increasing donor pool source. Transplantation 2000; 70:314-317.
46. Schold JD, Kaplan B, Howard RJ et al. Are we frozen in time? Analysis of the utilization and efficacy of pulsatile perfusion in renal transplantation. Am J Transplant 2005; 5:1681-1688.
47. Bond M, Pitt M, Akoh J et al. The effectiveness and cost-effectiveness of methods of storing donated kidneys from deceased donors: a systematic review and economic model. Health Technol Assess 2009; 13:iii-xiv, 1.
48. Moers C, Smits JM, Maathuis MH et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. N Engl J Med 2009; 360:7-19.
49. Pirenne J, Smits JM, Monbaliu D et al. Renal resistance during machine perfusion is a risk factor for delayed graft function and poorer graft survival. Transpl Int 2009; 22:56.
50. Moers C, Varnav OC, Treckmann J et al. GST and HFABP values during machine perfusion of deceased donor kidneys are independent predictors of delayed graft function, but not of primary non-function and graft survival. Transpl Int 2009; 22:42.
51. Kokkinos C, Antcliffe D, Nanidis T et al. Outcome of kidney transplantation from nonheart-beating versus heart-beating cadaveric donors. Transplantation 2007; 83:1193-1199.
52. Snoeijs MG, Schaefer S, Christiaans MH et al. Kidney transplantation using elderly non-heart-beating donors: a single-center experience. Am J Transplant 2006; 6:1066-1071.
53. Maheshwari A, Maley W, Li Z et al. Biliary complications and outcomes of liver transplantation from donors after cardiac death. Liver Transpl 2007; 13:1645-1653.
54. St Peter SD, Imber CJ, Friend PJ. Liver and kidney preservation by perfusion. Lancet 2002; 359:604-613.
55. Freeman RB, Jr., Steffick DE, Guidinger MK et al. Liver and intestine transplantation in the United States, 1997-2006. Am J Transplant 2008; 8:958-976.
56. Lee KW, Simpkins CE, Montgomery RA et al. Factors affecting graft survival after liver transplantation from donation after cardiac death donors. Transplantation 2006; 82:1683-1688.
57. Mateo R, Cho Y, Singh G et al. Risk factors for graft survival after liver transplantation from donation after cardiac death donors: an analysis of OPTN/UNOS data. Am J Transplant 2006; 6:791-796.
58. Egan TM. Non-heart-beating donors in thoracic transplantation. J Heart Lung Transplant 2004; 23:3-10.
59. Steen S, Sjoberg T, Pierre L et al. Transplantation of lungs from a non-heart-beating donor. Lancet 2001; 357:825-829.
60. Gómez de Antonio D, Marcos R, Laporta R et al. Results of clinical lung transplant from uncontrolled non-heart-beating donors. J Heart Lung Transplant 2007; 26:529-534.
61. Snell GI, Levvey BJ, Oto T et al. Early lung transplantation success utilizing controlled donation after cardiac death donors. Am J Transplant 2008; 8:1282-1289.

62. Mason DP, Murthy SC, Gonzalez-Stawinski GV et al. Early experience with lung transplantation using donors after cardiac death. J Heart Lung Transplant 2008; 27:561-563.
63. Fernandez LA, Di CA, Odorico JS et al. Simultaneous pancreas-kidney transplantation from donation after cardiac death: successful long-term outcomes. Ann Surg 2005; 242:716-723.
64. Markmann JF, Deng S, Desai NM et al. The use of non-heart-beating donors for isolated pancreatic islet transplantation. Transplantation 2003; 75:1423-1429.
65. Singhal AK, Abrams JD, Mohara J et al. Potential suitability for transplantation of hearts from human non-heart-beating donors: data review from the Gift of Life Donor Program. J Heart Lung Transplant 2005; 24:1657-1664.
66. Cobianchi L, Zonta S, Vigano J et al. Experimental small bowel transplantation from non-heart-beating donors: a large-animal study. Transplant Proc 2009; 41:55-56.
67. Koffman G, Gambaro G. Renal transplantation from non-heart- beating donors: a review of the European experience. J Nephrol 2003; 16:334-341.
68. Steinbrook R. Organ donation after cardiac death. N Engl J Med 2007; 19;357:209-213.
69. Ruggenenti P, Perico N, Remuzzi G. Ways to boost kidney transplant viability: a real need for the best use of older donors. Am J Transplant 2006; 6:2543-2547.
70. Quiroga I, McShane P, Koo DD et al. Major effects of delayed graft function and cold ischaemia time on renal allograft survival. Nephrol Dial Transplant 2006; 21:1689-1696.
71. Woo YM, Gill JS, Johnson N et al. The advanced age deceased kidney donor: current outcomes and future opportunities. Kidney Int 2005; 67:2407-2414.
72. Giblin L, O'Kelly P, Little D et al. A comparison of long-term graft survival rates between the first and second donor kidney transplanted--the effect of a longer cold ischaemic time for the second kidney. Am J Transplant 2005; 5:1071-1075.
73. Keith DS, DeMattos A, Golconda M et al. Factors associated with improvement in deceased donor renal allograft function in the 1990s. J Am Soc Nephrol 2005; 16:1512-1521.
74. Schold JD, Kaplan B, Baliga RS et al. The broad spectrum of quality in deceased donor kidneys. Am J Transplant 2005; 5:757-765.
75. Ploeg RJ, Visser MJ, Stijnen T et al. Impact of donor age and quality of donor kidneys on graft survival. Transplant Proc 1987; 19:1532-1534.
76. Fritsche L, Horstrup J, Budde K et al. Old-for-old kidney allocation allows successful expansion of the donor and recipient pool. Am J Transplant 2003; 3:1434-1439.
77. Cox DR. Regression models and life-tables. J R Stat Soc 1972; 34:187-202.
78. Smits JM, Persijn GG, van Houwelingen HC et al. Evaluation of the Eurotransplant Senior Program. The results of the first year. Am J Transplant 2002; 2:664-670.
79. Cecka JM, Cohen B, Rosendale J et al. Could more effective use of kidneys recovered from older deceased donors result in more kidney transplants for older patients? Transplantation 2006; 81:966-970.
80. Matas AJ, Delmonico FL. Transplant kidneys sooner: discard fewer kidneys. Am J Transplant 2001; 1:301-304.

81. Brook NR, Waller JR, Richardson AC et al. A report on the activity and clinical outcomes of renal non-heart beating donor transplantation in the United Kingdom. Clin Transplant 2004; 18:627-633.
82. Hattori R, Ono Y, Yoshimura N et al. Long-term outcome of kidney transplant using non-heart-beating donor: multicenter analysis of factors affecting graft survival. Clin Transplant 2003; 17:518-521.
83. Keizer KM, de Fijter JW, Haase-Kromwijk BJ et al. Non-heart-beating donor kidneys in the Netherlands: allocation and outcome of transplantation. Transplantation 2005; 79:1195-1199.
84. Delmonico FL, Burdick JF. Maximizing the success of transplantation with kidneys from older donors. N Engl J Med 2006; 354:411-413.
85. Frei U, Noeldeke J, hold-Fabrizii V et al. Prospective age-matching in elderly kidney transplant recipients--a 5-year analysis of the eurotransplant senior program. Am J Transplant 2008; 8:50-57.
86. Opelz G, Dohler B. Effect of human leukocyte antigen compatibility on kidney graft survival: comparative analysis of two decades. Transplantation 2007; 84:137-143.
87. Pascual J, Marcen R, Ortuno J. Clinical experience with everolimus (Certican) in elderly recipients: the "old-for-old" concept. Transplantation 2005; 79:S85-S88.
88. Segoloni GP, Messina M, Squiccimarro G et al. Preferential allocation of marginal kidney allografts to elderly recipients combined with modified immunosuppression gives good results. Transplantation 2005; 80:953-958.
89. Arns W, Citterio F, Campistol JM. 'Old-for-old'--new strategies for renal transplantation. Nephrol Dial Transplant 2007; 22:336-341.
90. Moers C, Leuvenink HG, Ploeg RJ. Donation after cardiac death: evaluation of revisiting an important donor source. Nephrol Dial Transplant 2010; 25:666-673.
91. Maathuis MH, Leuvenink HG, Ploeg RJ. Perspectives in organ preservation. Transplantation 2007; 83:1289-1298.
92. Garcia-Valdecasas JC, Fondevila C. In-vivo normothermic recirculation: an update. Curr Opin Organ Transplant 2010; 15:173-176.
93. Net M, Valero R, Almenara R et al. The effect of normothermic recirculation is mediated by ischemic preconditioning in NHBD liver transplantation. Am J Transplant 2005; 5:2385-2392.
94. Valero R, Cabrer C, Oppenheimer F et al. Normothermic recirculation reduces primary graft dysfunction of kidneys obtained from non-heart-beating donors. Transpl Int 2000; 13:303-310.
95. Maathuis MH, Ottens PJ, van GH et al. Static cold storage preservation of ischemically damaged kidneys. a comparison between IGL-1 and UW solution. Transpl Int 2008; 21:473-482.
96. Hoogland ER, Snoeijs MG, van Heurn LW. DCD kidney transplantation: results and measures to improve outcome. Curr Opin Organ Transplant 2010; 15:177-182.
97. Jochmans I, Moers C, Smits JM et al. Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death: a multicenter, randomized, controlled trial. Ann Surg 2010; 252:756-764.
98. Garcia-Valdecasas JC, Tabet J, Valero R et al. Liver conditioning after cardiac arrest: the use of normothermic recirculation in an experimental animal model. Transpl Int 1998; 11:424-432.
99. Schumacher M, Van Vliet BN, Ferrari P. Kidney transplantation in rats: an appraisal of surgical techniques and outcome. Microsurgery 2003; 23:387-394.

100. Kosieradzki M, Ametani M, Southard JH et al. Is ischemic preconditioning of the kidney clinically relevant? Surgery 2003; 133:81-90.
101. Dittrich S, Groneberg DA, von Loeper J et al. Influence of cold storage on renal ischemia reperfusion injury after non-heart-beating donor explantation. Nephron Exp Nephrol 2004; 96:e97-102.
102. Tilney NL, Guttmann RD. Effects of initial ischemia/reperfusion injury on the transplanted kidney. Transplantation 1997; 64:945-947.
103. Ploeg RJ, van Bockel JH, Langendijk PT et al. Effect of preservation solution on results of cadaveric kidney transplantation. The European Multicentre Study Group. Lancet 1992; 340:129-137.
104. Rutten FF, Ploeg RJ, McDonnell J et al. The cost-effectiveness of preservation with UW and EC solution for use in cadaveric kidney transplantation in the case of single kidney donors. Transplantation 1993; 56:854-858.
105. Wight J, Chilcott J, Holmes M et al. The clinical and cost-effectiveness of pulsatile machine perfusion versus cold storage of kidneys for transplantation retrieved from heart-beating and non-heart-beating donors. Health Technol Assess 2003; 7:1-94.
106. Wight JP, Chilcott JB, Holmes MW et al. Pulsatile machine perfusion vs. cold storage of kidneys for transplantation: a rapid and systematic review. Clin Transplant 2003; 17:293-307.
107. Oosterlee A, Rahmel A, van Zwet W. Eurotransplant Annual Report. Eurotransplant International Foundation, Leiden, The Netherlands 2006.
108. Belzer FO, Glass NR, Sollinger HW et al. A new perfusate for kidney preservation. Transplantation 1982; 33:322-323.
109. Boom H, Paul LC, de Fijter JW. Delayed graft function in renal transplantation. Transplantation Reviews 2004; 18:139-152.
110. Hsieh FY, Bloch DA, Larsen MD. A simple method of sample size calculation for linear and logistic regression. Stat Med 1998; 17:1623-1634.
111. Daly PJ, Power RE, Healy DA et al. Delayed graft function: a dilemma in renal transplantation. BJU Int 2005; 96:498-501.
112. McLaren AJ, Jassem W, Gray DW et al. Delayed graft function: risk factors and the relative effects of early function and acute rejection on long-term survival in cadaveric renal transplantation. Clin Transplant 1999; 13:266-272.
113. Troppmann C, Gillingham KJ, Benedetti E et al. Delayed graft function, acute rejection, and outcome after cadaver renal transplantation. The multivariate analysis. Transplantation 1995; 59:962-968.
114. Buyse ME, Staquet MJ, Sylvester RJ. Cancer clinical trials - methods and practice. Oxford University Press; 1992.
115. Wang R, Lagakos SW, Ware JH et al. Statistics in medicine--reporting of subgroup analyses in clinical trials. N Engl J Med 2007; 357:2189-2194.
116. Daemen JH, de Vries B, Oomen AP et al. Effect of machine perfusion preservation on delayed graft function in non-heart-beating donor kidneys--early results. Transpl Int 1997; 10:317-322.
117. Kwiatkowski A, Wszola M, Kosieradzki M et al. Machine perfusion preservation improves renal allograft survival. Am J Transplant 2007; 7:1942-1947.

118. Matsuno N, Kozaki K, Degawa H et al. A useful predictor in machine perfusion preservation for kidney transplantation from non-heart-beating donors. Transplant Proc 2000; 32:173-174.
119. Merion RM, Oh HK, Port FK et al. A prospective controlled trial of cold-storage versus machine-perfusion preservation in cadaveric renal transplantation. Transplantation 1990; 50:230-233.
120. van der Vliet JA, Kievit JK, Hene RJ et al. Preservation of non-heart-beating donor kidneys: a clinical prospective randomised case-control study of machine perfusion versus cold storage. Transplant Proc 2001; 33:847.
121. Saidi RF, Elias N, Kawai T et al. Outcome of kidney transplantation using expanded criteria donors and donation after cardiac death kidneys: realities and costs. Am J Transplant 2007; 7:2769-2774.
122. Matsuno N, Konno O, Mejit A et al. Application of machine perfusion preservation as a viability test for marginal kidney graft. Transplantation 2006; 82:1425-1428.
123. Halloran PF, Hunsicker LG. Delayed graft function: state of the art, November 10-11, 2000. Summit meeting, Scottsdale, Arizona, USA. Am J Transplant 2001; 1:115-120.
124. Kauffman HM, McBride MA, Cors CS et al. Early mortality rates in older kidney recipients with comorbid risk factors. Transplantation 2007; 83:404-410.
125. Yarlagadda SG, Coca SG, Formica RN, Jr. et al. Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. Nephrol Dial Transplant 2009; 24:1039-1047.
126. Hagenmeyer EG, Haussler B, Hempel E et al. Resource use and treatment costs after kidney transplantation: impact of demographic factors, comorbidities, and complications. Transplantation 2004; 77:1545-1550.
127. Rosenthal JT, Danovitch GM, Wilkinson A et al. The high cost of delayed graft function in cadaveric renal transplantation. Transplantation 1991; 51:1115-1118.
128. Kootstra G, van Heurn E. Non-heartbeating donation of kidneys for transplantation. Nat Clin Pract Nephrol 2007; 3:154-163.
129. Daemen JW, Oomen AP, Kelders WP et al. The potential pool of non-heart-beating kidney donors. Clin Transplant 1997; 11:149-154.
130. Moustafellos P, Hadjianastassiou V, Roy D et al. The influence of pulsatile preservation in kidney transplantation from non-heart-beating donors. Transplant Proc 2007; 39:1323-1325.
131. Plata-Munoz JJ, Muthusamy A, Quiroga I et al. Impact of pulsatile perfusion on postoperative outcome of kidneys from controlled donors after cardiac death. Transpl Int 2008; 21:899-907.
132. Opelz G, Terasaki PI. Advantage of cold storage over machine perfusion for preservation of cadaver kidneys. Transplantation 1982; 33:64-68.
133. Ojo AO, Wolfe RA, Held PJ et al. Delayed graft function: risk factors and implications for renal allograft survival. Transplantation 1997; 63:968-974.
134. Brook NR, Waller JR, Nicholson ML. Nonheart-beating kidney donation: current practice and future developments. Kidney Int 2003; 63:1516-1529.
135. Barklin A. Systemic inflammation in the brain-dead organ donor. Acta Anaesthesiol Scand 2009; 53:425-435.

136. Rowinski W, Walaszewski J, Lagiewska B et al. Use of kidneys from marginal and non-heart-beating donors: warm ischemia per se is not the most detrimental factor. Transplant Proc 1993; 25:1511-1512.
137. Daemen JH, de Vries B, Kootstra G. The effect of machine perfusion preservation on early function of non-heart-beating donor kidneys. Transplant Proc 1997; 29:3489.
138. Watson CJ, Wells AC, Roberts RJ et al. Cold machine perfusion versus static cold storage of kidneys donated after cardiac death: a UK multicenter randomized controlled trial. Am J Transplant 2010; 10:1991-1999.
139. Clarke A, Rosen R. Length of stay. How short should hospital care be? Eur J Public Health 2001; 11:166-170.
140. Morgan M, Beech R. Variations in lengths of stay and rates of day case surgery: implications for the efficiency of surgical management. J Epidemiol Community Health 1990; 44:90-105.
141. Stewart ZA, Lonze BE, Warren DS et al. Histidine-tryptophan-ketoglutarate (HTK) is associated with reduced graft survival of deceased donor kidney transplants. Am J Transplant 2009; 9:1048-1054.
142. Pascual J, Zamora J, Pirsch JD. A systematic review of kidney transplantation from expanded criteria donors. Am J Kidney Dis 2008; 52:553-586.
143. Port FK, Bragg-Gresham JL, Metzger RA et al. Donor characteristics associated with reduced graft survival: an approach to expanding the pool of kidney donors. Transplantation 2002; 74:1281-1286.
144. Ojo AO, Hanson JA, Meier-Kriesche H et al. Survival in recipients of marginal cadaveric donor kidneys compared with other recipients and wait-listed transplant candidates. J Am Soc Nephrol 2001; 12:589-597.
145. Polyak MM, Arrington BO, Stubenbord WT et al. The influence of pulsatile preservation on renal transplantation in the 1990s. Transplantation 2000; 69:249-258.
146. Moore J, Tan K, Cockwell P et al. Predicting early renal allograft function using clinical variables. Nephrol Dial Transplant 2007; 22:2669-2677.
147. Buchanan PM, Lentine KL, Burroughs TE et al. Association of lower costs of pulsatile machine perfusion in renal transplantation from expanded criteria donors. Am J Transplant 2008; 8:2391-2401.
148. Matsuoka L, Shah T, Aswad S et al. Pulsatile perfusion reduces the incidence of delayed graft function in expanded criteria donor kidney transplantation. Am J Transplant 2006; 6:1473-1478.
149. Stratta RJ, Moore PS, Farney AC et al. Influence of pulsatile perfusion preservation on outcomes in kidney transplantation from expanded criteria donors. J Am Coll Surg 2007; 204:873-882.
150. Jochmans I, Moers C, Smits JM et al. The Prognostic Value of Renal Resistance During Hypothermic Machine Perfusion of Deceased Donor Kidneys. Am J Transplant 2011.
151. Hosgood SA, Yang B, Bagul A et al. A comparison of hypothermic machine perfusion versus static cold storage in an experimental model of renal ischemia reperfusion injury. Transplantation 2010; 89:830-837.
152. Polyak M, Boykin J, Arrington B et al. Pulsatile preservation characteristics predict early graft function in extended criteria donor kidneys. Transplant Proc 1997; 29:3582-3583.
153. Polyak MM, Arrington BO, Kapur S et al. Calcium ion concentration of machine perfusate predicts early graft function in expanded criteria donor kidneys. Transpl Int 1999; 12:378-382.

154. Yuan X, Theruvath AJ, Ge X et al. Machine perfusion or cold storage in organ transplantation: indication, mechanisms, and future perspectives. Transpl Int 2010; 23:561-570.
155. Nyberg SL, Baskin-Bey ES, Kremers W et al. Improving the prediction of donor kidney quality: deceased donor score and resistive indices. Transplantation 2005; 80:925-929.
156. Buxton MJ, Drummond MF, Van Hout BA et al. Modelling in economic evaluation: an unavoidable fact of life. Health Econ 1997; 6:217-227.
157. de Wit GA, Ramsteijn PG, de Charro FT. Economic evaluation of end stage renal disease treatment. Health Policy 1998; 44:215-232.
158. Briggs AH, Wonderling DE, Mooney CZ. Pulling cost-effectiveness analysis up by its bootstraps: a non-parametric approach to confidence interval estimation. Health Econ 1997; 6:327-340.
159. Efron B, Tibshirani RJ. An introduction to the bootstrap. In: Chapman & Hall, New York; 1993.
160. Watson CJE, Wells AC, Roberts RJ et al. Cold Machine Perfusion Versus Static Cold Storage of Kidneys Donated After Cardiac Death: A UK Multicenter Randomized Controlled Trial. American Journal of Transplantation 2010; 10:1991-1999.
161. Liem YS, Wong JB, Hunink MG et al. Comparison of hemodialysis and peritoneal dialysis survival in The Netherlands. Kidney Int 2007; 71:153-158.
162. Hilbrands LB, Hoitsma AJ, Koene KA. Randomized, prospective trial of cyclosporine monotherapy versus azathioprine-prednisone from three months after renal transplantation. Transplantation 1996; 61:1038-1046.
163. Brooks R. EuroQol: the current state of play. Health Policy 1996; 37:53-72.
164. The Euroqol Group. EuroQol--a new facility for the measurement of health-related quality of life. Health Policy 1990; 16:199-208.
165. Torrance GW, Thomas WH, Sackett DL. A utility maximization model for evaluation of health care programs. Health Serv Res 1972; 7:118-133.
166. Churchill DN, Torrance GW, Taylor DW et al. Measurement of quality of life in end-stage renal disease: the time trade-off approach. Clin Invest Med 1987; 10:14-20.
167. Dolan P, Gudex C, Kind P et al. The time trade-off method: results from a general population study. Health Econ 1996; 5:141-154.
168. Dolan P. Modelling valuations for health states: the effect of duration. Health Policy 1996; 38:189-203.
169. Bremer BA, McCauley CR, Wrona RM et al. Quality of life in end-stage renal disease: a reexamination. Am J Kidney Dis 1989; 13:200-209.
170. Laupacis A, Keown P, Pus N et al. A study of the quality of life and cost-utility of renal transplantation. Kidney Int 1996; 50:235-242.
171. Evans RW, Manninen DL, Garrison LP, Jr. et al. The quality of life of patients with end-stage renal disease. N Engl J Med 1985; 312:553-559.
172. Daemen JW, Oomen AP, Janssen MA et al. Glutathione S-transferase as predictor of functional outcome in transplantation of machine-preserved non-heart-beating donor kidneys. Transplantation 1997; 63:89-93.
173. Gok MA, Pelzers M, Glatz JF et al. Do tissue damage biomarkers used to assess machine-perfused NHBD kidneys predict long-term renal function post-transplant? Clin Chim Acta 2003; 338:33-43.

174. Gok MA, Pelsers M, Glatz JF et al. Use of two biomarkers of renal ischemia to assess machine-perfused non-heart-beating donor kidneys. Clin Chem 2003; 49:172-175.
175. Balupuri S, Talbot D, El Sheikh M et al. Comparison of proteolytic enzymes and glutathione S-transferase levels in non-heart-beating donors' (NHBD) kidney perfusates. Clin Chem Lab Med 2000; 38:1099-1102.
176. Kovacevic Z, McGivan JD. Mitochondrial metabolism of glutamine and glutamate and its physiological significance. Physiol Rev 1983; 63:547-605.
177. Beckett GJ, Hayes JD. Glutathione S-transferases: biomedical applications. Adv Clin Chem 1993; 30:281-380.
178. Loeb WF. The measurement of renal injury. Toxicol Pathol 1998; 26:26-28.
179. Kotanko P, Margreiter R, Pfaller W. Urinary N-acetyl-beta-D-glucosaminidase and neopterin aid in the diagnosis of rejection and acute tubular necrosis in initially nonfunctioning kidney grafts. Nephron 2000; 84:228-235.
180. Veerkamp JH, van Moerkerk HT. Fatty acid-binding protein and its relation to fatty acid oxidation. Mol Cell Biochem 1993; 123:101-106.
181. Pelsers MM, Hermens WT, Glatz JF. Fatty acid-binding proteins as plasma markers of tissue injury. Clin Chim Acta 2005; 352:15-35.
182. Rao PS, Schaubel DE, Guidinger MK et al. A comprehensive risk quantification score for deceased donor kidneys: the kidney donor risk index. Transplantation 2009; 88:231-236.
183. Moore J, Ramakrishna S, Tan K et al. Identification of the optimal donor quality scoring system and measure of early renal function in kidney transplantation. Transplantation 2009; 87:578-586.
184. Murugan R, Venkataraman R, Wahed AS et al. Increased plasma interleukin-6 in donors is associated with lower recipient hospital-free survival after cadaveric organ transplantation. Crit Care Med 2008; 36:1810-1816.
185. Daemen JH, Heineman E, Kootstra G. Viability assessment of non-heart-beating donor kidneys during machine preservation. Transplant Proc 1995; 27:2906-2907.
186. Pepe MS. The statistical evaluation of medical tests for classification and prediction. Oxford, UK: Oxford University Press; 2003.
187. van den Eijnden MM, Leuvenink HG, Ottens PJ et al. Effect of brain death and non-heart-beating kidney donation on renal function and injury: an assessment in the isolated perfused rat kidney. Exp Clin Transplant 2003; 1:85-95.
188. Findlay J, Levvy GA, Marsh CA. Inhibition of glycosidases by aldonolactones of corresponding configuration. 2. Inhibitors of beta-N-acetylglucosaminidase. Biochem J 1958; 69:467-476.
189. Pfleiderer G. Particle bound aminopeptidase from pig kidney. In: Methods in enzymology. London: Academic Press; 1970:514-521.
190. Henry ML, Sommer BG, Ferguson RM. Renal blood flow and intrarenal resistance predict immediate renal allograft function. Transplant Proc 1986; 18:557-558.
191. Sampson D, Jun HM, Walczak P. Flow and function in machine-preserved kidneys. Br J Surg 1978; 65:37-40.

192. Sonnenday CJ, Cooper M, Kraus E et al. The hazards of basing acceptance of cadaveric renal allografts on pulsatile perfusion parameters alone. Transplantation 2003; 75:2029-2033.
193. Mozes MF, Skolek RB, Korf BC. Use of perfusion parameters in predicting outcomes of machine-preserved kidneys. Transplant Proc 2005; 37:350-351.
194. Guarrera JV, Goldstein MJ, Samstein B et al. 'When good kidneys pump badly': outcomes of deceased donor renal allografts with poor pulsatile perfusion characteristics. Transpl Int 2010; 23:444-446.
195. Yushkov YY, Alvarez-Casas J, Dikman S et al. Machine measured renal resistance (MMRR) is the most sensitive tool for prediction of early renal allograft survival. Transplantation 2010; 90:290.
196. Mancini GB, Dahlof B, Diez J. Surrogate markers for cardiovascular disease: structural markers. Circulation 2004; 109:IV22-IV30.
197. Irish WD, McCollum DA, Tesi RJ et al. Nomogram for predicting the likelihood of delayed graft function in adult cadaveric renal transplant recipients. J Am Soc Nephrol 2003; 14:2967-2974.
198. Irish WD, Ilsley JN, Schnitzler MA et al. A risk prediction model for delayed graft function in the current era of deceased donor renal transplantation. Am J Transplant 2010; 10:2279-2286.
199. Moers C, Varnav OC, van Heurn E et al. The value of machine perfusion perfusate biomarkers for predicting kidney transplant outcome. Transplantation 2010; 90:966-973.
200. Gok MA, Asher JF, Shenton BK et al. Graft function after kidney transplantation from non-heartbeating donors according to maastricht category. J Urol 2004; 172:2331-2334.
201. Ekberg H, Tedesco-Silva H, Demirbas A et al. Reduced exposure to calcineurin inhibitors in renal transplantation. N Engl J Med 2007; 357:2562-2575.
202. Bagul A, Hosgood SA, Kaushik M et al. Experimental renal preservation by normothermic resuscitation perfusion with autologous blood. Br J Surg 2008; 95:111-118.
203. Metcalfe MS, Waller JR, Hosgood SA et al. A paired study comparing the efficacy of renal preservation by normothermic autologous blood perfusion and hypothermic pulsatile perfusion. Transplant Proc 2002; 34:1473-1474.
204. St Peter SD, Imber CJ, Lopez I et al. Extended preservation of non-heart-beating donor livers with normothermic machine perfusion. Br J Surg 2002; 89:609-616.
205. Belzer FO, Ashby BS, Dunphy JE. 24-hour and 72-hour preservation of canine kidneys. Lancet 1967; 2:536-538.
206. Damman J, Nijboer WN, Schuurs TA et al. Local renal complement C3 induction by donor brain death is associated with reduced renal allograft function after transplantation. Nephrol Dial Transplant 2011; 26:2345-2354.
207. Jochmans I, Moers C, Ploeg RJ et al. To perfuse or not to perfuse kidneys donated after cardiac death. Am J Transplant 11 A.D.; 11:409-410.
208. Barnett D. Machine perfusion systems and cold static storage of kidneys from deceased donors. 165. 2010. London, United Kingdom, National Institute for Health and Clinical Excellence. NICE Technology Appraisal Guidance.
209. Gok MA, Pelsers M, Glatz JF et al. Creatinine clearance and viability biomarkers of machine perfused non-heart-beating donors: is there a correlation? Transplant Proc 2003; 35:768.

SUMMARY

Chapter 1 provides a brief general introduction to the thesis, in which the various types of organ donors are introduced and in **chapter 2** the practise of donation after cardiac death is discussed in detail. Transplant centers worldwide are revisiting donation after cardiac death as a tool to enlarge the deceased donor pool. This chapter reviews mechanisms of warm ischemic injury, potential new approaches to improve posttransplant results, and several persistently controversial issues that are pertinent to this type of organ donation. In addition, it provides an overview of current protocols for donation after cardiac death and up-to-date evidence on selection criteria, organ preservation, and clinical outcome after transplantation of various types of organs that are recovered from such donors.

Chapter 3 presents the results of a retrospective study in the large database of the American Organ Procurement and Transplantation Network, which evaluated the effect of deceased donor age on outcome after renal transplantation. Regression models which are relevant to guide clinical organ allocation policies were constructed and we simulated the effect that old-for-old allocation would have on transplant outcome. The analyses showed that donor age strongly influences posttransplant results, not only in the upper extremes, but for the whole range of donor ages ≥11. Furthermore, we found that implementation of old-for-old kidney allocation is likely to be safe. Such a policy could reduce waiting time for aged candidates, but according to our simulation it will not necessarily improve overall kidney transplant outcome.

Chapter 4 describes an animal study in which the concept of normothermic recirculation is investigated as a potential tool to improve the quality of kidneys that are severely damaged by warm ischemia. In this study, we subjected Lewis rat kidneys to various amounts of warm ischemia, and subsequently to different time periods of normothermic recirculation. After cold storage these kidneys were transplanted into recipient animals and 24 h later we measured the percentage of cortical necrosis, and determined the expression of several important genes that are involved in renal damage, inflammation, interstitial fibrosis formation, cytoprotection, and tissue regeneration. We found that normothermic recirculation had no significant influence on any of these markers. Hence, we concluded that these preclinical data by no means support the presumed beneficial effect of normothermic recirculation on kidneys that have sustained profound warm ischemic injury.

Chapter 5 is the main report of the Machine Preservation Trial. This international randomized controlled trial compared hypothermic machine perfusion with static cold storage for the preservation of deceased donor kidneys. In The Netherlands, Belgium, and the federal state of North-Rhine Westphalia in Germany, kidney pairs from 336 consecutive deceased

donors were enrolled. One kidney of each pair was randomly assigned to machine perfusion and the other to cold storage. Kidneys could be transplanted in the whole Eurotransplant region and recipients were followed up until 1 year after transplantation. We found that machine perfusion significantly reduced the risk of delayed graft function in all common types of deceased donor kidneys. In addition, machine perfusion was associated with a reduced risk of graft failure, and 1-year allograft survival was superior in the machine perfusion group.

Chapter 6 reports the results of a pre-specified sub-study of the Machine Preservation Trial. After an extended enrollment period, 82 kidney pairs from consecutive, controlled donors after cardiac death were included. One kidney was randomly assigned to machine perfusion, and the contralateral organ to static cold storage. As in the main trial, machine perfusion significantly reduced the incidence of delayed graft function. However, for kidneys donated after cardiac death 1-year graft survival was similar in both treatment groups.

Chapter 7 presents another pre-specified sub-study of the Machine Preservation Trial. Ninety-one kidney pairs from consecutive, expanded criteria donors after brain death were included and studied as a separate sub-group. One kidney was randomly assigned to machine perfusion, the contralateral organ to static cold storage. In these data, machine perfusion also significantly reduced the risk of delayed graft function compared with cold storage. The incidence of primary non-function in the cold storage group was four times higher than in the machine perfusion group, and 1-year graft survival was significantly better in machine perfused kidneys. In those patients who developed delayed graft function, 1-year graft survival was remarkably superior when their transplanted kidney had been machine perfused (85% vs. 41%).

Chapter 8 concisely describes the results of our 3-year follow-up analysis of the Machine Preservation Trial. As the original study had found an important 1-year graft survival advantage for machine perfused kidneys, we decided to investigate whether this advantage would persist 3 years posttransplant. We found that graft survival of kidneys donated after brain death remained significantly better after machine perfusion compared to cold storage, especially in kidneys recovered from expanded criteria donors. Delayed graft function was associated with a notably lower graft survival of kidneys donated after brain death. Despite the large reduction in delayed graft function by machine perfusion in kidneys donated after cardiocirculatory death that we showed earlier, there was no positive effect of machine perfusion on graft survival in this sub-group.

Chapter 9 is a cost-effectiveness analysis of machine perfusion versus cold storage based on data of the Machine Preservation Trial. This economic evaluation combined short term results derived from the empirical data in the clinical study with a Markov model that had a 10-year time horizon. The short-term evaluation showed that machine perfusion reduced the

risk of delayed graft function and graft failure at lower costs than cold storage. The Markov model revealed that life-years and QALYs can be gained while reducing costs at the same time, when kidneys are preserved by machine perfusion instead of cold storage.

Chapter 10 shows an analysis of whether six different biomarkers that were measured in the perfusate during machine perfusion have predictive value for outcome after deceased-donor kidney transplantation. From 306 deceased donor kidneys that were included in the Machine Preservation Trial, we tested whether concentrations were independently associated with delayed graft function, primary non-function, and graft survival. Three biomarkers proved to be independent – albeit mediocre – predictors of delayed graft function, but not of primary non-function and graft survival. The other three markers had no independent prognostic potential for any of the end points. We concluded that, although elevated concentrations of certain biomarkers during machine perfusion may be an indication to adjust posttransplant recipient management, the values of these markers alone should not lead to kidney discard.

Chapter 11 is the report of another analysis that evaluated the pre-transplant predictive potential of measurements during organ preservation. In this study, we investigated the prognostic value of renal intravascular resistance during machine perfusion on delayed graft function, primary non-function and graft survival of deceased donor kidneys. This sub-study of the Machine Preservation Trial showed that primary non-functioning kidneys had renal resistances which were comparable to those that showed immediate or delayed graft function. Renal resistance at the end of machine perfusion was an independent risk factor for delayed graft function. The best threshold value for predicting this end point had a high negative predictive value but a poor discriminative capacity. This study demonstrated that measurement of renal resistance during machine perfusion can be a valuable additional tool to predict aspects of transplant outcome, but renal resistance values alone should not be used to discard kidneys for transplantation.

NEDERLANDSE SAMENVATTING

Niertransplantatie is de beste medische behandeling voor patiënten die lijden aan eindstadium nierfalen. De afgelopen decennia is de samenstelling van de overleden donorpool radicaal veranderd, zodanig dat er steeds meer organen beschikbaar komen van oudere donoren, die vaak al meerdere aandoeningen in de voorgeschiedenis hebben, of van zogenaamde non-heart beating donoren bij wie de orgaanuitname pas kan beginnen wanneer het hart al enkele minuten stil heeft gestaan. Zulke orgaandonoren worden ook wel marginale donoren genoemd, omdat hun organen in het algemeen van minder goede kwaliteit zijn in vergelijking met die van jonge, verder gezonde hersendode donoren.

Dit proefschrift beschrijft de resultaten van klinische en pre-klinische studies op het gebied van niertransplantatie. In deze studies wordt de invloed die verscheidene karakteristieken van overleden orgaandonoren op het transplantatieresultaat hebben gekwantificeerd. Tevens wordt het effect onderzocht van interventies vóór of gedurende orgaanpreservatie, die zijn gericht op het beter conserveren van de orgaankwaliteit voorafgaand aan de transplantatie. Daarnaast beschrijft het proefschrift een studie waarin biomarkers worden gemeten in de orgaanpreservatievloeistof en een andere studie waarin de vasculaire weerstand wordt bepaald tijdens machinale preservatie van donornieren. Deze beide studies hebben als doel het voorspellen van de vitaliteit en de functie van het orgaan na transplantatie. Hoewel de resultaten van de studies in dit proefschift betrekking hebben op nieren afkomstig van alle typen overleden donoren, zijn ze het meest relevant voor marginale donornieren. Aangezien de functie en levensduur van zulke nieren na transplantatie vaak suboptimaal zijn, is extra informatie over hun kwaliteit nog vóór transplantatie belangrijk. Tevens zijn nieuwe interventies die de orgaanfunctie ná transplantatie verbeteren noodzakelijk.

Hoofdstuk 1 is een beknopte algemene inleiding op dit proefschrift, waarin de verschillende typen orgaandonoren worden geïntroduceerd en in **hoofdstuk 2** wordt de praktijk van orgaandonatie na hartdood (in Nederland meestal non-heart beating donatie genoemd) in detail besproken. In veel transplantatiecentra wereldwijd is non-heart beating donatie opnieuw ingevoerd in een poging om de overleden donorpool te vergroten. Dit hoofdstuk bespreekt de mechanismen van warme ischemieschade, nieuwe methoden om het transplantatieresultaat te verbeteren en relevante controverses die direct samenhangen met dit type orgaandonatie. Daarnaast wordt er een overzicht gegeven van bestaande protocollen voor non-heart beating donatie en van de meest actuele onderzoeksresultaten op het gebied van selectiecriteria, orgaanpreservatie en klinische uitkomsten na transplantatie van meerdere typen organen die afkomstig zijn van zulke donoren.

Hoofdstuk 3 bespreekt de resultaten van een retrospectieve studie in de grote database van het Amerikaanse Organ Procurement and Transplantation Network, waarin de invloed

van de leeftijd van overleden donoren op het transplantatieresultaat werd onderzocht. Er werden regressiemodellen vervaardigd die kunnen helpen om de orgaan-allocatie te sturen en we simuleerden het effect dat old-for-old allocatie zou kunnen hebben op het transplantatieresultaat. De analyses toonden aan dat de donorleeftijd een zeer grote invloed heeft op het transplantatieresultaat, niet alleen in de extreme leeftijdscategoriën, maar voor het hele spectrum van donorleeftijden van 11 jaar en ouder. Voorts berekenden we dat de implementatie van old-for-old allocatie waarschijnlijk veilig is. Een dergelijk beleid zou mogelijk de wachttijd voor oudere transplantatiekandidaten kunnen verkorten, maar onze simulatie liet ook zien dat deze aanpak niet vanzelfsprekend leidt tot een algehele verbetering van het transplantatieresultaat voor ontvangers van alle leeftijden samen.

Hoofdstuk 4 beschrijft een dierexperimenteel onderzoek waarin normotherme recirculatie werd bestudeerd, een potentieel nieuwe methode om de orgaankwaliteit van ernstig ischemisch beschadigde donornieren te verbeteren. In deze studie werden nieren van Lewis ratten blootgesteld aan verschillende hoeveelheden warme ischemie en vervolgens aan verschillende periodes van normotherme recirculatie. Na koude statische orgaanpreservatie werden deze nieren getransplanteerd in ontvangerdieren en na 24 uur overleving maten we het percentage corticale necrose in de getransplanteerde nier en werd de expressie van verscheidene belangrijke genen bepaald die betrokken zijn bij nierschade, ontsteking, vorming van interstitiële fibrose, cytoprotectie en weefselregeneratie. We ontdekten dat normotherme recirculatie geen significante invloed heeft op al deze variabelen. Daarom concludeerden we dat deze preklinische resultaten op geen enkele manier het vermeende positieve effect van normotherme recirculatie op ernstig ischemisch beschadigde donornieren bevestigen.

Hoofdstuk 5 rapporteert de belangrijkste bevindingen van de Machine Preservation Trial. In deze internationale gerandomiseerde studie werd hypotherme machineperfusie vergeleken met statisch koud bewaren (cold storage) voor het preserveren van nieren afkomstig van overleden donoren. In Nederland, België en Nordrhein-Westfalen in Duitsland werden paren nieren van 336 overleden donoren geïncludeerd. Eén nier van ieder paar werd willekeurig toegewezen aan machineperfusie en de andere nier werd statisch koud bewaard. Trialnieren konden worden getransplanteerd in de hele Eurotransplant-regio. De ontvangers van deze organen werden gedurende 1 jaar gevolgd. Uit de studie bleek dat machineperfusie het risico op delayed graft function (het vertraagd op gang komen van een donornier) significant vermindert voor alle veel voorkomende typen donornieren. Bovendien was machineperfusie geassocieerd met een lager risico op transplantaatfalen en was de 1-jaars transplantaatoverleving superieur in de machineperfusie groep.

Hoofdstuk 6 beschrijft de resultaten van een protocollair geplande sub-studie van de Machine Preservation Trial. Tijdens een verlengde inclusie-periode werden 82 opeenvolgende

paren non-heart beating donornieren gerandomiseerd. Eén nier werd machinaal gepreserveerd en de contralaterale nier werd statisch koud bewaard. Machineperfusie reduceerde ook bij non-heart beating donornieren de incidentie van delayed graft function. In tegenstelling tot de algemene resultaten die worden beschreven in hoofdstuk 5, was de 1-jaars transplantaatoverleving voor non-heart beating nieren niet verschillend in beide studie-armen.

Hoofdstuk 7 rapporteert eveneens resultaten van een protocollair geplande sub-studie van de Machine Preservation Trial. Eén-en-negentig paren donornieren afkomstig van hersendode zogenaamde expanded criteria donoren (donoren van hoge leeftijd en/of met significante comorbiditeit) werden geïncludeerd en bestudeerd als een aparte subgroep. Eén nier werd willekeurig toegewezen aan machineperfusie, de contralaterale nier werd statisch koud bewaard. Ook in deze studie bleek machineperfusie het risico op delayed graft function significant te verlagen in vergelijking met statisch koud bewaren. De incidentie van primary non-function (het nooit op gang komen van een getransplanteerde nier) was in de cold storage groep viermaal hoger dan in de machineperfusie groep en de 1-jaars transplantaatoverleving was significant beter voor machinaal gepreserveerde nieren. Bij patiënten die na transplantatie delayed graft function ontwikkelden, bleek de 1-jaars transplantaatoverleving opmerkelijk veel hoger indien hun donornier machinaal gepreserveerd was (85% vs. 41%).

Hoofdstuk 8 geeft een beknopt verslag van de 3-jaars follow-up van de Machine Preservation Trial. Aangezien de studies in hoofdstuk 5 en 7 een belangrijke verbetering in 1-jaars transplantaatoverleving als gevolg van machineperfusie hadden laten zien, besloten we te onderzoeken of dit effect na 3 jaar nog steeds aantoonbaar zou zijn. In deze analyse bleek de transplantaatoverleving na machineperfusie in vergelijking met statisch koud bewaren ook 3 jaar na transplantatie significant beter voor nieren afkomstig van hersendode donoren, met name voor die organen die afkomstig waren van expanded criteria donoren. Het ontwikkelen van delayed graft function was geassocieerd met een opvallend slechtere 3-jaars transplantaatoverleving voor nieren afkomstig van hersendode donoren. Ondanks de grote reductie van de kans op delayed graft function die we eerder vonden voor non-heart beating nieren, bleek er ook na 3 jaar geen positief effect te zijn van machineperfusie op de transplantaatoverlevning van dit type donororganen.

Hoofdstuk 9 is een kosteneffectiviteitsanalyse gebaseerd op data afkomstig van de Machine Preservation Trial, waarin machineperfusie werd vergeleken met statisch koud bewaren. Deze economische studie combineerde de korte termijn resultaten van de klinische trial met een Markov model met een tijdshorizon van 10 jaar na transplantatie. De korte termijn evaluatie toonde aan dat machineperfusie het risico op delayed graft function en transplantaatfalen reduceert en resulteert in lagere kosten vergeleken met statisch koud bewaren. Het Markov model liet zien dat levensjaren en QALY's toenemen terwijl

tegelijkertijd de totale kosten afnemen indien donornieren worden gepreserveerd door middel van machineperfusie in plaats van cold storage.

Hoofdstuk 10 beschrijft een studie waarin werd onderzocht of zes verschillende biomarkers, gemeten in het perfusaat tijdens machinale preservatie, een voorspellende waarde hebben voor klinische uitkomstmaten na transplantatie van nieren afkomstig van overleden donoren. Van 306 donornieren die waren geïncludeerd in de Machine Preservation Trial onderzochten we of de concentraties in het perfusaat onafhankelijk geassocieerd waren met delayed graft function, primary non-function en de transplantaatoverleving. Drie biomarkers bleken onafhankelijke, maar zwakke, voorspellers van delayed graft function, maar niet van primary non-function en de transplantaatoverleving. De andere drie biomarkers hadden geen onafhankelijke voorspellende waarde voor de uitkomstmaten van deze studie. We concludeerden dat, hoewel verhoogde perfusaat-concentraties van sommige biomarkers tijdens machinale preservatie een indicatie kunnen zijn tot het aanpassen van het vroege klinische beleid voor de ontvanger, dergelijke biomarkers nooit mogen worden betrokken bij beslissingen over het al dan niet accepteren van een donornier.

Hoofdstuk 11 geeft de bevindingen weer van een tweede analyse waarbij de voorspellende waarde van metingen tijdens orgaanpreservatie werd bestudeerd. In deze studie onderzochten we de prognostische waarde van de renale vasculaire weerstand tijdens machineperfusie voor delayed graft function, primary non-function en de transplantaatoverleving bij nieren afkomstig van overleden donoren. Deze sub-studie van de Machine Preservation Trial liet zien dat nieren die primary non-function vertonen vasculaire weerstanden tijdens machineperfusie hebben die vergelijkbaar zijn met zowel nieren die delayed graft function ontwikkelen als nieren die direct functioneren na transplantatie. De renale vasculaire weerstand aan het einde van de machinale preservatie bleek een onafhankelijke voorspeller voor het ontwikkelen van delayed graft function. De optimale drempelwaarde van de vasculaire weerstand voor het voorspellen van delayed graft function had weliswaar een hoge negatief voorspellende waarde, maar een slecht onderscheidend vermogen. Deze studie toonde aan dat het meten van de renale vasculaire weerstand tijdens machinale preservatie een waardevol extra instument kan zijn om aspecten van het transplantatieresultaat te voorspellen, maar dat dergelijke weerstandsmetingen op zichzelf niet mogen worden gebruikt voor de beslissing tot het wel of niet accepteren van een donornier.

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Cyril Moers werd op 21 maart 1979 geboren in Wenen, Oostenrijk. Hij volgde het gymnasium van het Bernardinuscollege te Heerlen en behaalde daar in 1997 cum laude zijn eindexamen. In 1998 rondde hij de propedeuse Sterrenkunde af aan de Rijksuniversiteit Groningen en in datzelfde jaar werd hij ingeloot voor de studie Geneeskunde aan de RUG. Hij deed zijn afstudeeronderzoek bij het Chirurgisch Onderzoekslaboratorium van het Universitair Medisch Centrum Groningen naar geïsoleerde dubbele perfusie van rattenlevers. Tijdens dat onderzoek werd zijn interesse gewekt voor de wetenschap in het algemeen en de orgaantransplantatie in het bijzonder. Hij volgde de reguliere coschappen in het UMCG en deed daarna een keuzecoschap bij de afdeling Chirurgie van het Martini Ziekenhuis te Groningen. In 2005 behaalde hij zijn artsenbul cum laude en begon hij als arts-onderzoeker aan een promotietraject bij het Chirurgisch Onderzoekslaboratorium, met als promotor prof. dr. R.J. Ploeg en copromotor dr. H.G.D. Leuvenink. In 2008 startte hij met de opleiding tot chirurg in het UMCG en inmiddels volgt hij de tweede helft van zijn opleiding in het Martini Ziekenhuis met als opleider dr. P.C. Baas. Cyril is getrouwd met Kirsten Heineman en in het voorjaar van 2011 werd hun dochter Roos geboren.

LIST OF PUBLICATIONS

't Hart NA, van der Plaats A, **Moers C**, et al. Development of the isolated dual perfused rat liver model as an improved reperfusion model for transplantation research. Int J Artif Organs 2006; 29(2):219-27.

Moers C, Leuvenink HGD, Ploeg RJ. Non heart beating organ donation – Overview and future perspectives. Transpl Int 2007;20(7):567-75.

Maathuis MHJ, **Moers C**, Leuvenink HGD, Ploeg RJ. Het succes van transplantatie begint met orgaanpreservatie. Ned Tijdschr Geneeskd 2008;152(13):737-41.

Moers C, Smits JM, Maathuis MHJ, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. N Engl J Med 2009;360(1):7-19.

Moers C, Kornmann NSS, Leuvenink HGD, Ploeg RJ. The influence of deceased donor age and old-for-old allocation on kidney transplant outcome. Transplantation 2009;88(4):542-52.

Moers C, Leuvenink HGD, Ploeg RJ. Donation after cardiac death: evaluation of revisiting an important donor source. Nephrol Dial Transplant 2010;25(3):666-73.

Moers C, Varnav OC, van Heurn LWE, et al. The value of machine perfusion perfusate biomarkers for predicting kidney transplant outcome. Transplantation 2010;90(9):966-73.

Jochmans I, **Moers C**, Smits JM, et al. Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death – a multicenter randomised controlled trial. Ann Surg 2010;252(5):756-64.

Nijboer WN, **Moers C**, Leuvenink HGD, et al. How important is the duration of the brain death period for the outcome in kidney transplantation? Transpl Int 2011;24(1):14-20.

Jochmans I, **Moers C**, Ploeg RJ, Pirenne J. To perfuse or not to perfuse kidneys donated after cardiac death. Am J Transplant 2011;11(2):409-10.

Treckmann J, **Moers C**, Smits JM, et al. Machine perfusion versus cold storage for preservation of kidneys from expanded criteria donors after brain death: results of a multi-center randomized trial. Transpl Int 2011;24(6):548-54.

Damman J, Seelen MA, **Moers C**, et al. Systemic complement activation in deceased donors is a predictor of acute rejection after renal transplantation. Transplantation 2011;92(2):163-69.

Jochmans I, **Moers C**, Smits JM, et al. The prognostic value of renal resistance during hypothermic machine perfusion of deceased donor kidneys. Am J Transplant 2011;11(10):2214-20.

Moers C, van Rijt G, Ploeg RJ, et al. The effect of normothermic recirculation before cold preservation on posttransplant injury of ischemically damaged donor kidneys. Transpl Int 2011; In press.

Varnav OC, **Moers C**, van Oeveren W, et al. The release of heart-type fatty acid binding protein and total glutathione S-transferase in donor plasma and in machine perfusion perfusate of brain dead and controlled non-heart-beating donor kidneys - What can we learn from it? *Submitted for publication*.

Groen H, **Moers C**, Smits JM, et al. Cost-effectiveness of hypothermic machine preservation versus static cold storage in renal transplantation. *Submitted for publication*.

Koudstaal LG, **Moers C**, Burgerhof JGM, et al. Donor serum angiopoietin 2 is an independent predictor of kidney transplant outcome. *Submitted for publication*.

Moers C, Pirenne J, Paul A, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation – three-year follow-up. *Submitted for publication*.